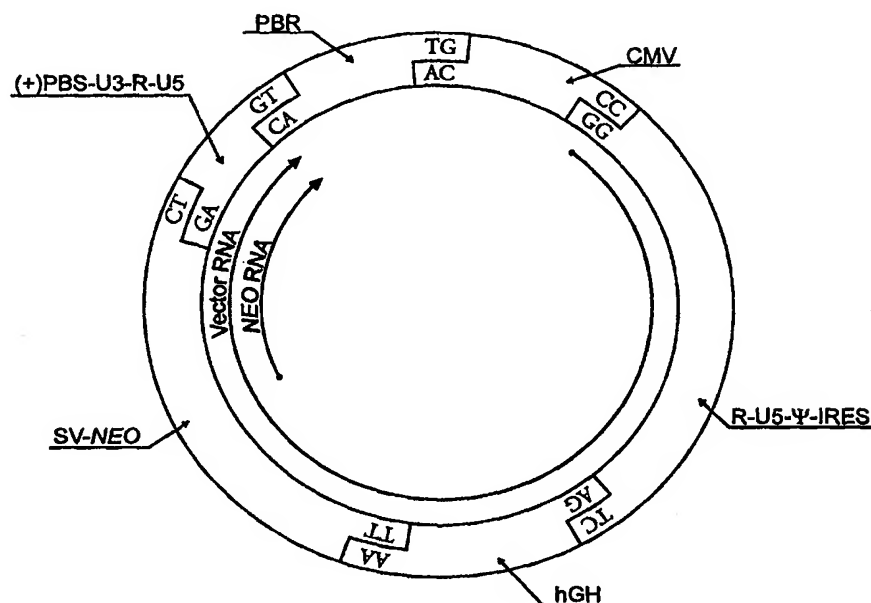




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/86, 15/10</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/38326</b> <b>(43) International Publication Date:</b> 3 September 1998 (03.09.98)
<b>(21) International Application Number:</b> PCT/US98/03918 <b>(22) International Filing Date:</b> 28 February 1998 (28.02.98)  <b>(30) Priority Data:</b> 60/070,910      28 February 1997 (28.02.97)      US  <b>(71) Applicant (for all designated States except US):</b> NATURE TECHNOLOGY CORPORATION [US/US]; 109 South 54th Street, Omaha, NE 68132 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> HODGSON, Clague, P. [US/US]; 109 South 54th Street, Omaha, NE 68132 (US). ZINK, Mary, Ann [US/US]; 109 South 54th Street, Omaha, NE 68132 (US). XU, Guoping [CN/US]; 109 South 54th Street, Omaha, NE 68132 (US).  <b>(74) Agent:</b> MCCORMACK, Myra, H.; Mueting, Raasch & Gebhardt, P.A., P.O. Box 581415, Minneapolis, MN 55458-1415 (US).		<b>(81) Designated States:</b> AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

**(54) Title:** SELF-ASSEMBLING GENES, VECTORS AND USES THEREOF

**(57) Abstract**

The invention relates to a method for directing the self-assembly of a gene or gene assembly having three and preferably six or more fragments in a directionally and spatially ordered fashion to produce a gene, gene vector or large nucleic acid molecule. The method can be used to create libraries, such as combinatorial libraries. In another embodiment of the invention a vector is described for the incorporation and screening of endogenous mouse promoter elements for the identification of cell-specific promoters.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece			<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>ML</b>	Mali	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MN</b>	Mongolia	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MR</b>	Mauritania	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MW</b>	Malawi	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>MX</b>	Mexico	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NE</b>	Niger	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NL</b>	Netherlands	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NO</b>	Norway	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>NZ</b>	New Zealand		
<b>CM</b>	Cameroon			<b>PL</b>	Poland		
<b>CN</b>	China	<b>KR</b>	Republic of Korea	<b>PT</b>	Portugal		
<b>CU</b>	Cuba	<b>KZ</b>	Kazakstan	<b>RO</b>	Romania		
<b>CZ</b>	Czech Republic	<b>LC</b>	Saint Lucia	<b>RU</b>	Russian Federation		
<b>DE</b>	Germany	<b>LI</b>	Liechtenstein	<b>SD</b>	Sudan		
<b>DK</b>	Denmark	<b>LK</b>	Sri Lanka	<b>SE</b>	Sweden		
<b>EE</b>	Estonia	<b>LR</b>	Liberia	<b>SG</b>	Singapore		

## SELF-ASSEMBLING GENES, VECTORS AND USES THEREOF

### Field of the Invention

5           This invention relates to the construction and usage of synthetic genes for genetic engineering and gene therapy.

### Background of the invention

10           This application claims the benefit of a provisional application U.S. Serial No. 60/070,910, filed on February 28, 1997, entitled "Self-Assembling Genes."

15           Recombination at the genetic level is important for generating diversity and adaptive change within genomes of virtually all organisms. Recombinant DNA technology is based upon simple 'cut-and-paste' methods for manipulating nucleic acid molecules *in vitro*. The pieces of genetic material, or DNA are first digested with a restriction endonuclease enzyme which recognizes specific sequences within the DNA. After preparation of two or more pieces of DNA, the ends of the DNA are further manipulated, if necessary, to make them compatible for ligation or joining together. DNA ligase, together with adenosine triphosphate (ATP) is added to the genes, ligating them back together. The genetic assembly containing an origin of DNA replication and a selectable gene is then inserted into a living cell, is grown up, and is positively selected to yield a pure culture capable of providing high yields of individual recombinant DNA molecules, or their products such as RNA or protein.

20           Significant improvements have been made to this technology over the last two and a half decades. Numerous enzymes, end-linkers and adapter molecules have been made commercially available, which facilitate in the construction of recombinant DNA molecules. By using two restriction enzymes with different single-stranded termini or blunt ends, it is possible to directionally assemble genes (forced cloning). This reduces the amount of screening required to determine orientation. Procedures have been automated for synthesis of single-stranded gene fragments up to 200 or more nucleotides in length by means of phosphoramidite chemistry, and the instrumentation is readily available through Applied Biosystems, Inc., Foster City, CA. Such single-stranded fragments can be joined by annealing overlapping complimentary phosphorylated strands, and by enzymatically filling in the ends with DNA polymerase and DNA precursors. In this way, multiple, overlapping, single-stranded fragments can be assembled into a larger, double-stranded superstructure.

Whole genes have been synthesized by similar methods. However, it becomes increasingly difficult to use synthetic DNA strands when making genes larger than approximately one kilobase. Using gene amplification methods (e.g. polymerase chain reaction (PCR), Mullis *et al.*, U.S. Patent 4,683,195), together with synthetic oligonucleotides, it is possible to make  
5 biologically active, synthetic retro-vectors that are capable of RNA transcription, reverse-transcription, viral packaging, and integration into genomic DNA (see for example, Hodgson, WO94/20608). Hodgson, *supra*, also disclosed methods for cloning of transcriptional promoters into such a vector using traditional recombinant DNA technology.

Modified restriction enzyme sites, linkers, and adapters can change the  
10 primary or secondary structure of complex nucleic acid sequences thereby altering or obliterating a desired biological activity. For example, small mutations can drastically modify transcriptional promoters or change the reading frame of coding DNA. A logical goal of vectorology is to make exact constructs, without need of fortuitous restriction sites, adapters, or linkers.

15 Restriction endonucleases can be grouped based on similar characteristics. In general there are three major types or classes: I, II (including IIS) and III. Class I enzymes cuts at a somewhat random site from the enzyme recognition sites (see Old and Primrose, 1994. *Principles of Gene Manipulation*. Blackwell Sciences, Inc., Cambridge, MA, p.24). Most enzymes used in molecular biology are type II enzymes. These enzymes recognize a  
20 particular target sequence (i.e., restriction endonuclease recognition site) and break the polynucleotide chains within or near to the recognition site. The type II recognition sequences are continuous or interrupted. Class IIS enzymes (i.e., type IIS enzymes) have asymmetric recognition sequences. Cleavage occurs at a distance from the recognition site.

These enzymes have been reviewed by Szybalski et al. *Gene* 100:13-26, 1991. Class  
25 III restriction enzymes are rare and are not commonly used in molecular biology.

U.S. Patent No. 4,293,652 employed a linker with a class IIS enzyme recognition sequence to permit synthesized DNA to be inserted into a vector without disturbing a recognition sequence. Brousseau et al. (*Gene* 17:279-289, 1982) and Urdea et al. (*Proc. Natl. Acad. Sci. USA* 80:7461-7465, 1983) disclose the use of class IIS enzymes for  
30 the production of vectors to produce recombinant insulin and epidermal growth factor respectively. Mandecki et al. described a method for making synthetic genes by cloning small oligonucleotides using a vector (*Gene* 68:101-107, 1988). Expansion of a population of



oligonucleotides required synthesis, cloning excision and fragment purification. The oligonucleotides were used to create a complete plasmid.

Lebedenko et al. (*Nucl. Acids Res.* 19(24):6757-6771) illustrated the class IIS enzymes and PCR for precisely joining 3 nucleic acid molecules for convention sub-cloning using BamHI. Tomic et al. (*Nucleic Acids Res.*, 18:1656, 1990), reported a method for site-directed mutagenesis using the polymerase chain reaction and class IIS enzymes to join two nucleic acid molecules. Two overlapping PCR primers were used where the primers included class IIS recognition sites. The primers included a region of complementarity to the template DNA and include one to a few site-directed mutations. Stemmer et al. (U.S. Patent No. 5,514,568) employed overlapping primers with class IIS enzymes to amplify a plasmid and to introduce specific mutations into DNA leaving all other positions unaltered.

There remains a need for the ordering and assembly of complex genes to overcome the problems associated with sequential sub-cloning such as multiple purification steps, the potential for sample loss, and the like. Moreover there is a need for eliminating the use of prokaryotic hosts and for minimizing or avoiding the risks associated with bacterial contamination resulting from the use of bacteria as intermediaries in the cloning process. Further, there remains a need for efficient methods to assemble large nucleic acid molecules or many-fragmented nucleic acid assemblies with precision.

### Brief Description of the Figures

**Fig. 1A.** provides one schematic of six double stranded DNA fragments, each terminus comprising a unique overhanging two-nucleotide sequence complementary to only one other terminus

**Fig. 1B.** illustrates a three-piece ligation where 100% of the clones tested contained the predicted fragment order and desired fragment orientation.

**Fig. 2.** illustrates the use of a class IIS restriction endonuclease (as one example, *Bpm1*), restriction endonuclease recognition site and the selection of cohesive overhanging ends.

**Fig. 3A.** illustrates an exemplary retrotransposon-derived vector including a murine VL30 LTR (NLV-3) and packaging signal, an internal ribosome entry site (IRES) from encephalomyocarditis virus (EMCV), a gene encoding a green fluorescent protein (GFP), additional internal VL30 sequences (solid bar), SV40 early region promoter and Tn5

aminoglycosidase phosphotransferase (neo) gene, PBR322 plasmid origin of replication and a plus-strand primer binding site (VL30). An exemplary vector sequence is provided as VLBPGN (SEQ ID NO:1). Fig 3B is an illustration of an LTR with the insertion of a U3 (transcriptional promoter) region rescued by reverse transcriptase-polymerase chain reaction (RT-PCR). The promoter is amplified from the RNA of a cell expressing the VL30 U3 region. Complementary overhanging ends are created using class IIS restriction endonuclease digestion sites within the LTR and within the promoter. Fig. 3C provides the linear structure of a VL30 RNA transcript from a mouse cell with a U3 region near the 3'-terminus of the RNA molecule. PCR primers include a class IIS enzyme recognition site to amplify the U3 region from the RNA resulting in a double stranded DNA molecule. Cleavage with a class IIS enzyme (here *BpmI*), results in a double-stranded DNA molecule with end complementary to a site in the vector of Fig. 3A.

**Fig. 4A.** is a schematic illustrating steps for assembling a combinatorial library of *cis*- or *trans*-acting nucleic acid sequences for assembly and screening, useful for the rescue of biologically active species. Fig. 4b is a diagram of a U3 (transcriptional enhancer and promoter region of an LTR illustrating several sub-divisions of the transcriptional control region, including a distal enhancer region, an enhancer repeat region, a medial promoter and a proximal promoter. These regions have been described for other vectors in Hodgson et al. (1996. "Construction, Transmission and Expression of Synthetic VL30 Vectors" in Hodgson ed. *Retro-vectors for Human Gene Therapy*. RG Landes Company, Austin TX). Segments of these regions are amplified using primers for highly conserved sequences. Highly conserved sequences are determine based on a comparison of known VL30 sequences such as provided in Fig. 4.2 of Hodgson, 1996, *infra*). The parts are joined by annealing and ligation to provide an ordered assembly. Each construct is an allele or a representative of allelic variation in the combinatorial library.

**Fig. 5** discloses two transcriptional promoters that have been rescued from mouse VL30 RNA sequences isolated from a mouse T-helper cell library. These promoters were assembled into a vector and introduced into retroviral helper cells and packaged into recombinant retrovirus for introduction into human T-cells. After transduction to human T cells, a  $\beta$ -galactosidase reporter gene was expressed from the T cell-derived promoters.

**Fig. 6** discloses 10 biologically active mouse VL30 promoters obtained from mouse liver RNA. These promoters were introduced into the vector of SEQ ID NO:1. The vectors

were introduced into retroviral helper cells and then packaged into retrovirus where they were introduced into human liver cells. The cells expressed the green fluorescent protein.

**Fig. 7** illustrates a similarity plot of nucleotide sequences found in VL30 U3 regions.

**Fig. 8** illustrates a retro-vector comprising six double-stranded DNA fragments that were self-assembled into a circular structure using unique overlapping termini created using class IIS restriction endonucleases. Three templates and twelve primers were used in conjunction with three class IIS enzymes to make the six fragments that were ligated in a single step. The vector was efficiently self-assembled and was effectively transmitted by both DNA transfection as well as by retroviral transduction of the self-assembled DNA, without molecular cloning through a prokaryotic host (see Example 2).

### BRIEF SUMMARY OF THE INVENTION

The invention described herein provides seamless, directional, ordered construction of complex DNA molecules, vectors and libraries. More particularly, it enables gene constructs to be assembled with greater efficiency and precision, and it enables multiple gene fragments to be assembled in the correct order and orientation without disturbing the internal structure of the gene. The method utilizes *in vitro* assembly of nucleic acid fragments and relies upon the unusual ability of certain enzymes to digest nucleic acid molecules at pre-determined sites without disrupting the structure of the gene. It is especially useful for the construction of genetic vectors for gene therapy or genetic engineering of cells and organisms. A particular application of the invention is in combinatorial, or evolutionary genetics, where it enables a large number of non-random, self-assembled constructs to be screened simultaneously for function.

In a preferred embodiment of this invention, the invention relates to a method for assembling a gene or gene vector comprising the steps of: a) designing at least 6 primers to produce to amplify at least three fragments in at least three separate polymerase chain reactions wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template nucleic acid for amplification, and bases positioned at the restriction endonuclease cleavage site that are selected to be complementary to only one other overhanging created from enzymatic cleavage of the fragments; b) combining the primers with template nucleic acid and performing the

polymerase chain reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site; c) digesting the amplified template fragments with one or more restriction endonucleases that recognize the restriction endonuclease recognition site of the primers to create overhanging termini wherein each overhanging termini is complementary to only one other overhanging termini on another fragment; and d) combining the amplified and digested template fragments in a ligation reaction to produce a directionally ordered gene, nucleic acid fragment or gene vector.

In a preferred aspect of this embodiment, the restriction endonuclease is at least one class IIS restriction endonuclease and preferably, the class IIS restriction endonuclease is selected from the group consisting of: *AlwI*, *Alw26I*, *BbsI*, *BbvI*, *BbvII*, *BpmI*, *BsmAI*, *BsmI*, *BsmBI*, *BspMI*, *BsrI*, *BsrDI*, *Eco57I*, *EarI*, *FokI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MnlII*, *PleI*, *SapI*, *SfaNI*, *TaqII*, *Tth111II*. Still more preferably, class II restriction endonuclease recognition sites (to be distinguished from class IIS restriction endonuclease recognition sites), linkers, or adapters are not used to create the gene or gene vector. In one embodiment, the product of the ligation reaction is introduced into prokaryotic or eukaryotic cells. Preferably, at least one template nucleic acid sequence is chosen from the group consisting of : transcriptional regulatory sequences; genetic vectors; introns and/or exons; viral encapsidation sequences; integration signals intended for introducing nucleic acid molecules into other nucleic acid molecules; retrotransposon(s); VL30 elements; or multiple allelic forms of a sequence.

In another preferred aspect of this embodiment, the method is used to generate combinatorial libraries of a target sequence. Preferably, the target sequence is part or all of a gene. In one embodiment, the gene encodes a protein. In one embodiment, the primers amplify allelic variants of part or all of a gene.

In still another preferred aspect of this embodiment, the product of the ligation reaction is passed between eukaryotic cells using a virus particle, by cell fusion, or by transfection. Preferably the product of the ligation reaction is not introduced into prokaryotic cells. Moreover, the method further comprises combining at least one screening or selection step to select the products of the ligation reaction. In one embodiment, the product of the ligation reaction is mutated during passage in cells in order to generate genetic diversity and preferably the product of the ligation reaction is mutated by homologous recombination during passage in cells.

In another aspect of this embodiment, the method is used to isolate and identify regulatory sequences from a cell. In another aspect of this embodiment, cells containing the product of the ligation reaction are selected for enhanced biological activity. Preferably, the cells containing the product of the ligation reaction are selected for tissue-specific, hormone-specific or developmental-specific gene expression. Also preferably, the ligation reaction is a circularized gene vector.

In another embodiment of this invention, the invention relates to a nucleic acid primer having a 5' and a 3' end to amplify a nucleic acid fragment for the ligation of at least two fragments comprising: a restriction endonuclease recognition site that recognizes a restriction endonuclease, wherein the restriction endonuclease cleaves at a distance from the recognition site and creates overhanging termini; a sequence complementary to a template sequence to be amplified to produce the nucleic acid fragment; at least two nucleic acid bases positioned at the restriction endonuclease cleavage site and that form an overhanging terminus after cleavage by the restriction endonuclease, wherein the at least two nucleic acid bases are selected to be complementary to only one other overhanging terminus on another fragment of the ligation; and an affinity handle on the 5' end of the primer. Preferably the primer further comprises an anchor to provide stability to the restriction enzyme at the restriction enzyme recognition site.

In yet another embodiment of this invention, the invention relates to a method for isolating and identifying promoters comprising the steps of: a) obtaining a vector comprising at least a portion of a promoter region from a retrovirus transposon LTR and having two non-complementary overhanging termini; b) designing at least two PCR primers to amplify at least one region of a retrovirus transposon LTR from template nucleic acid to produce at least one nucleic acid fragment wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template sequence from a retrovirus transposon, and bases positioned at the restriction endonuclease cleavage site that are selected to be complementary to only one other overhanging terminus of the vector wherein the restriction endonuclease cleavage site is created from enzymatic cleavage of the fragments; b) combining the primers with template nucleic acid and performing a polymerase chain reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site; c)

digesting the amplified template fragments with one or more restriction endonuclease that recognize the restriction endonuclease recognition site of the primer to create overhanging termini; and combining the amplified and digested template fragment in a ligation reaction with the vector to produce a gene vector with an intact LTR sequence. In one embodiment of this aspect of the invention, the template nucleic acid is DNA or RNA. In another embodiment of this aspect of the invention, the method further comprises the step of sequencing the insert to identify the promoter sequence. In one embodiment promoter sequences of SEQ ID NOS:1-13 identified using the methods of claim.

### **Detailed Description of the Invention**

In one embodiment of this invention, the invention relates to the seamless, oriented self-assembly of at least three DNA fragments having overlapping unique cohesive ends generated by the enzymatic cleavage of at least one restriction endonuclease that is capable of cleaving at a site distant to the restriction enzyme recognition site. Preferably the restriction endonucleases employed in this invention are class IIS restriction endonucleases. These enzymes recognize a predetermined group of nucleotides and cleave at a distance characteristic of the particular endonuclease from the recognition site. The term "unique cohesive ends" is used herein to refer to the notion that the cleavage site for the endonucleases of this invention can be manipulated to produce overhanging ends with unique termini selected by the investigator. The term "complementary" as used herein in reference to the overhanging ends of the fragments of this invention refers to standard complementarity recognized in the field of molecular biology. For example, the nucleotides sequence 5'-TAG-3' is said to be complementary to the nucleotide sequence 5'-CTA-3'. The term "PCR" is used generally to refer to the polymerase chain reaction and its variations, including RT-PCR as well as other gene amplification techniques employing primers.

In a first step for practicing one embodiment of this invention, a series of at least three overlapping fragments are created through the selection and creation of primers incorporating at least one class IIS restriction enzyme recognition sequence. The oligonucleotide primers of this invention are designed to amplify one or more nucleic acid fragments and comprise a sequence complementary to a target sequence for gene amplification, a recognition sequence for a restriction endonuclease that cleaves DNA at a distance from the recognition sequence (such as a class IIS restriction enzyme) and bases

positioned at the restriction endonuclease cleavage site that are preferably unique and complementary to only one other overhanging termini in the annealing/ligation reaction that generates the complex nucleic acid molecules. Optionally, the primers of this invention can include an "affinity handle for cleanup" at the 5' end. These sequences can be of any length, preferably at least about 6 bp and the sequences extend the primer in the 5' direction from the restriction enzyme recognition site. This extra length gives many enzymes greater stability and improved activity. In addition, the sequence can be used for recognition and removal of the ends of the primers (either undigested fragments or digested ends of primers) using complementary nucleotide sequences bound to a solid support (such as cellulose, nitrocellulose or silica). Incubation with, or passage over a column or support containing the complementary sequences can be used to remove the tags by allowing them to anneal or hybridize. The nucleic acid can then be eluted from the column. Adapters can also be used in this invention. For purposes of this invention, adapters refer to double stranded fragments containing an enzyme recognition site, according to this invention. The adapters are ligated to double stranded DNA molecules, creating a fragment analogous to a PCR fragment with similar sites derived from a primer. The primers or adapters can be prepared using a number of methods for synthesizing oligonucleotides known in the art. For example instruments for producing oligonucleotides are available from Applied Biosystems, Inc., Foster City, CA.

In one example, for the design of an oligonucleotide primer for use in this invention, the particular complementary bases that will form the site for hybridization of the primer to template (i.e., target DNA or RNA) are selected. A restriction endonuclease recognition site is selected followed by a number of nucleotides to be positioned between the recognition site and the cleavage site. The nucleotides of the cleavage site are selected to include overhanging regions formed from the restriction endonuclease cleavage that are complementary to the overhanging regions of an adjacent fragment in the annealing/ligation reaction.

The length of the primer used in this invention can vary, but preferably the primer length is up to about 80 bases and preferably up to about 50 bases. In addition the primers are preferably at least about 15 bases in length and preferably at least about 25 bases in length. The 5' region of the primer contains preferably at least about 6, preferably at least about 10 and still more preferably at least about 16-18 bases that are not complementary to the template DNA or RNA. Further, the primer incorporates a restriction endonuclease

recognition site preferably 5' to the region of complementarity and a restriction endonuclease digestion site preferably 5' to the region of complementarity or within the region of complementarity. There are a variety of restriction endonucleases that cleave at a distance from the restriction endonuclease recognition site of a DNA strand and a variety of enzymes that are commercially available from New England Biolabs are provided in Table 1.

**Table 1. Restriction endonucleases useful in the construction of self-assembling genes**

Enzyme:	Site size (bp):	Distance to overlap:	Size of overlap:	Overlap type:
<i>Alw26 I</i>	5	1-5bp	4bp	5'-Overhang
<i>BbsI</i>	6	2-6bp	4bp	5'-overhang
<i>BpmI</i>	6	16-14bp	2bp	3'-overhang
<i>BsmBI</i>	6	1-5bp	4bp	5'-overhang
<i>BspMI</i>	6	4-8bp	4bp	5'-overhang
<i>BsrDI</i>	6	0-2bp	2bp	3'-overhang
<i>Eco57I</i>	6	16-14bp	2bp	3'-overhang
<i>FokI</i>	5	9-13bp	4bp	5'-overhang
<i>HgaI</i>	5	5-10bp	5bp	5'-overhang
<i>HphI</i>	5	8-7bp	1bp	3'-overhang
<i>MnII</i>	5	7-6bp	1bp	3'-overhang
<i>PleI</i>	5	4-5bp	1bp	5'-overhang
<i>SapI</i>	7	1-4bp	3bp	5'-overhang
<i>SfaNI</i>	5	5-9bp	4bp	5'-overhang

In addition to the enzymes provided in Table 1, other restriction endonucleases that cleave at a distance from their restriction endonuclease recognition site include, but are not limited to, *AlwI*, *BbsI*, *BbvI*, *BbvII*, *BsmAI*, *BsmI*, *BsrI*, *EarI*, *GsuI*, *MboII*, *TaqII*, *Tth111III* and their respective isoschizomers. These and other enzymes are known in the art and many are available from other manufacturers. The primers can be prepared to produce either 5'-overlapping ends or 3'-overlapping ends, as long as they are both are either 5'-overlapping ends or 3'-overlapping ends and are complementary to one other set of overlapping ends.

In the case of *BpmI* (see Example 1), the enzyme digests asymmetrically, 14-16 bp from the 3'-nucleotide of the recognition site. The resulting cleavage has a 3'-overhanging end of 2 bp. A second primer is then designed with a complementary



overhanging end, and it is used to generate the adjoining fragment terminus. At the opposite ends of the two fragments that are to be joined, similar complementary, overhanging ends are designed.

The oligonucleotides are then combined with template nucleic acid (either  
5 DNA or RNA, e.g., such as for reverse transcriptase polymerase chain reaction (RT-PCR))  
containing bases complementary to at least a 3' portion of the primers (also referred to herein  
as "templates"). In one embodiment, the fragments are gene-amplified by PCR, RT-PCR or  
another gene amplification process using established PCR protocols such as those provided  
with PCR amplification kits, including those available from Perkin-Elmer Corp. (Emeryville,  
10 California). Preferably, the PCR products are analyzed by electrophoresis on a gel, such as  
an agarose gel and still more preferably the fragments of the predicted size are purified free of  
excess primers and small byproducts (such as by purification through a small column, such as  
a Qiagen<sup>TM</sup> column (Qiagen, Valencia, CA)). Following amplification or purification, the  
fragments are digested with the restriction endonuclease recognizing the restriction  
15 endonuclease recognition site in the primers. The digested fragments are then purified from  
the digested ends of the primers, preferably by preparative agarose gel electrophoresis. The  
fragments are combined, annealed and are ligated using standard hybridization and ligation  
conditions known for cloning (see Ausubel et al., *Current Protocols in Molecular Biology*,  
John Wiley & Sons, 1994).

20 Fig. 1A illustrates an example of a self-assembling gene construct (SEQ ID  
NO:1) comprising six fragments, each having unique overhanging dinucleotide ends. In this  
example, the ends of the fragments prepared by the methods of this invention are constructed  
using primers that include *BpmI* restriction endonuclease recognition sites. It will be  
understood by those of ordinary skill in the art that one or more other restriction  
25 endonucleases (such as those of Table 1) could similarly be used for the self-assembling  
product of Fig. 1A. In a preferred embodiment, the primers were created as described above  
and preferably the 3' ends of the primers are non-palindromic (i.e., non self-complementary)  
to prevent self-annealing of such fragments. Each fragment in this example preferably joins to  
only one other dinucleotide overhang in the annealing/ligation mixture, assuring ligation only  
30 to the intended fragment partner. An advantage of this strategy is that the formation of  
concatamers or multimers is minimal. The restriction endonuclease site is removed by

digestion with the restriction endonuclease, leaving the junction free of the extra DNA sequences associated with the site.

Using a single restriction endonuclease with a dinucleotide overhang (for example, using the enzyme *BpmI*) up to six pieces of genetic material can be joined together in a linear or circular form (such as a vector) without the need to perform sub-cloning procedures or detailed analysis of individual products because six unique combinations of dinucleotide overhangs create a directional clone with extremely high fidelity. With enzymes digesting single-base overlaps, only two fragments can be joined with positional and directional precision. With enzymes digesting three-base overlaps,  $4^3/2$ , or 32 fragments can be so joined in the correct order and orientation. Therefore, this invention also relates to the use of restriction endonuclease recognition sites that facilitate cleavage by restriction endonucleases with three-base overlaps and self-assembly gene constructs including 32 fragments. Alternatively, a combination of restriction endonuclease recognition sites for use with a combination of restriction enzymes that create two-base or three-base overlaps can be used. Each enzyme has its characteristic limits to self-assembly imposed by the size of the overlap. For example, there are sixteen dinucleotides, therefore *BpmI* fragments (which have two dinucleotide ends each) are limited to eight for the purpose of self-assembly; therefore in another embodiment of this invention an assembly comprising eight fragments is contemplated. However, four of the sixteen dinucleotides are palindromes. Use of these palindromic dinucleotides can create some infidelity in the annealing/ligation reaction. The enzyme *HgaI* has a five base overlap, and there are 1,024 pentanucleotide combinations, permitting 512 fragments to be ligated together directionally and in order (no palindromes). The fragments to be joined at a particular place are designed to have their cut sites aligned, so that the overlapping region fits together. In some cases, the target sequences will contain natural restriction endonuclease recognition sites for the enzyme that is being used, such as one or more internal *BpmI* sites. These sites have the potential to self-religate during vector or gene construction or they can be by passed by using a substitute enzyme in the primers (for example, *Eco 571* can substitute for *BpmI*). Alternatively, these sites can be removed by site-directed mutagenesis after consideration to the consequences of the mutagenized sequence to the gene or vector.

In addition to class IIS enzymes, class II restriction endonucleases can be used. These enzymes have intrinsic methylation activity that affects the outcome in either a

negative or a positive way, depending on the purpose for which it is used. In a preferred embodiment, the methylation activity of class II enzymes is ablated by mutation or by genetic engineering to convert the enzyme to an effective class IIS enzyme to expand the repertoire of useful enzymes for this invention.

5                   In another aspect of this invention, the primer design and target fragment sequence selection can be automated (see Example 5) using a computer to assist in the selection of unique overhanging ends that have complementarity only to the overhanging end of an adjacent fragment.

10                   Therefore, this invention permits high-fidelity annealing and ligation of six or more fragments with unique overhanging termini complementary to a single other overhanging termini. Any multitude of combinations can be created by combining the type of overhanging termini that can be created. Moreover, if one is willing to sacrifice the fidelity of the reaction, a variety of combinations can be used to anneal a variety of fragment numbers. In these cases, some selection may be necessary, such as size selection of the  
15                   resulting fragment based on electrophoretic migration or restriction endonuclease profiling, both methods well known to those of ordinary skill in the art

                  It is also necessary to have a high per-step efficiency (e.g., each step in the process is performed with an efficiency of at least 80%) to effectively ligate large numbers of fragments without error. Where large numbers of fragments are used, the purity of the  
20                   fragments becomes important. This means that for large numbers of fragments, the digested DNA fragments for annealing and ligation should be substantially pure. If undigested fragments, digested ends of primers, degraded or partially degraded molecules are present, they can decrease the purity and affect the fidelity of the product. Therefore, it is particularly desirable to ensure complete digestion of both ends of each fragment and to remove all of the  
25                   digested ends from the fragments prior to including the fragments in an annealing and ligation reaction. The use of Qiagen columns for oligonucleotide removal prior to digestion is generally sufficient to permit efficient digestion of the fragments. Agarose gel isolation is desirable after digestion particularly where the product contains some fragments that do not appear to be full length. The use of an analytical gel before and after digestion helps in  
30                   determining whether both oligonucleotide tags have been removed. The isolation of fragments from agarose gels preferably avoids the use of ultraviolet light and exposure of the

DNA to ethidium bromide is also preferably avoided. These methods can be avoided by running replicate lanes and staining only a portion of the gel.

The fragments and vector are then digested to yield fully complementary ends, and the fragments are preferably again purified, as described above (such as through a Qiagen column or by gel isolation). The purified fragments are ligated together in a test tube, under standard conditions, such as using bacteriophage T4 DNA ligase and ATP. Preferred ligations include at least 20µg/ml total DNA concentration in the ligation mix to favor intermolecular interactions, and an equimolar ratio of fragments to be ligated. Where a prokaryotic intermediary is used, the ligated assemblage is transformed into a bacterium, such as an *E. coli* host, and the colonies are selected with a drug (such as an ampicillin, tetracycline, or kanamycin marker). The colonies can then be selected either by individually selecting colonies or growing a mass culture, such as where a vector library has been created. Restriction enzyme analysis can be used to determine the identity of individual constructs or to assess the validation of the combination of plasmids. The plasmids can then be grown up and used as needed.

In one embodiment of this invention, at least a portion of a vector is used as one of the fragments for the ligation of at least three fragments according to this invention. In one example, where a vector is used as one of the starting fragments, two restriction endonuclease recognition sites recognizing an enzyme that cleaves at a distance from the recognition site, such as at least one *BpmI* site, can also be introduced into the vector. This permits the vector to be digested with the restriction endonuclease to produce a product having ends complementary to two ends of the insert DNA fragments. The vector can be made by amplifying a plasmid or portion thereof using the primers of this invention. Thus, the vector can also be constructed to include a variety of restriction endonuclease recognition sites using a variety of restriction endonucleases, including a variety of class II restriction endonucleases. In some cases, the target fragments for amplification will contain natural restriction endonuclease recognition sites for the enzyme that is being used for the self-assembly, such as for example, a fragment that includes one or more internal *BpmI* sites. Care should be taken either to utilize the complementarity of the naturally occurring site to reform the fragment as it originally existed or to eliminate the restriction endonuclease recognition site using, for example, site-directed mutagenesis. Preferably, the restriction endonuclease recognition site is substituted for a different enzyme (in the case of *BpmI*,

substituting *Eco57I* or *BsrDI*) that has an equivalent structure at its ends. Two or more fragments of insert or two or more fragments of vector with at least one insert are amplified using primers according to this invention.

The exemplary enzyme, *BpmI* digests DNA 14-16 base pairs (bp) from the 3'-  
5 nucleotide of the recognition sequence (RS). Thus, by placing the RS exactly 14-16 bp from the desired dinucleotide cut site, the practitioner tags the dinucleotide for ligation with another dinucleotide that is exactly complementary to it. Such a complementary dinucleotide can be inserted by using the same enzyme and RS to make another fragment which fits the first exactly, as illustrated in Fig. 1. Because there are sixteen possible dinucleotide  
10 combinations (including twelve combinations that do not have palindromic ends), it is possible to create up to six fragments with unique dinucleotides, and it is also possible to join them all together in a predetermined order and orientation (Fig 1A). In addition, the palindromic sequences (such as AT, CG, TA, and GC) could also be used, although inefficiency and incorrect ligation will result from the self-complimentarity of these  
15 sequences. It is furthermore possible and desirable to have three or more fragments joined in this way, such that the construct is circular as in Fig. 1, comprising a vector that may be grown in a bacterial and/or eukaryotic host cell. If the genetic construct is to be used as a vector, the vector should be designed to include a proper origin of replication to enable it to replicate in a particular cell. For example, a prokaryotic origin of replication such as a  
20 coliform plasmid origin of replication enables circular DNAs to be propagated in *E. coli* host cells. It is desirable to have at least one selectable marker, such as a neomycin marker that enables recovery of the clone through a selection process. It is also desirable, but not essential, to have two or more selectable genetic elements, to permit dual selection. For example, if one of the fragments contains a prokaryotic plasmid origin of replication, and  
25 another fragment contains a selectable marker, then the two fragments are both selectable, since the construct will grow in prokaryotic cells in the presence of a selection drug (such as ampicillin) only when both fragments are present. Drug selection can be combined with the methods of directed self-assembly to assure a high percentage of correct products. Because of the unique complementarity of the fragments, each contributes a selectable element that  
30 leads to recovery of a high percentage of correct products.

For prokaryotic vector construction, at least one fragment should contain a prokaryotic origin of replication and one fragment should contain a drug resistance marker

gene. However, an advantage of the methods of this invention is that the construct can be introduced directly into eukaryotic cells. Here no plasmid origin of replication is necessary and no prokaryotic selectable marker or other prokaryotic nucleic acid sequence is necessary. In cases where the vector is subject to regulatory approval or where optimal gene function is necessary, it may be undesirable to include prokaryotic sequences, such as extraneous plasmids or expressed prokaryotic fragments particularly if the sequences contain immunostimulatory sites that can lead to activation of the intracellular immune system and inactivation of a gene product (see Krieg et al., *J. Lab. Clin. Med.*, 128:128-133, 1996) or to avoid risks of endotoxin contamination. Moreover, the use of self-assembled product, according to the methods of this invention saves labor and time involved in the screening process.

Thus, in a preferred embodiment of the invention, the nucleic acid fragments are self-assembled *in vitro*, and are transferred directly into eukaryotic cells, by transfection, injection, or other methods known in the art. In one embodiment the cells receiving the assembled product of this invention are helper cells for recombinant virus assembly (including, but not limited to retroviral helper cells for retroviral or retrotransposon vectors, adenovirus helper cells for adenovirus vectors or herpes simplex virus helper cells for herpes simplex vectors). Alternatively, the assembled product can be introduced into cells along with a helper virus or the assembled product can be introduced into target cells for direct expression. The assembled product can be a vector, a minichromosome vector, a portion of a chromosome, or the like. In the preferred case of a retroviral vector, the genes are first transfected into a first helper cell line (such as ecotropic helper cells, GP+E86 (Markowitz et al. *J. Virol.* 862:1120-1124, 1988). The retrovirus-containing supernatant from these cells is then filtered (0.45mm Nalgene filters) preferably 48-72 hours after transfection and the filtrate is transferred to a second complementation retroviral helper cell line (such as PA317 retroviral helper cells, Miller et al., *Mol. Cell. Biol.* 6:2895-2902, 1986). After an additional 48 h, the second helper cell line is selected with the marker drug (such as the drug G418 for the selectable neomycin (neo) marker gene), until only drug-resistant cells remain. These cells contain stably integrated vectors that can be used to repeatedly transduce human cells. Advantageously, in the case of adenovirus vectors or other large eukaryotic -derived vectors including eukaryotic virus-derived vectors, it may be impossible to propagate them in prokaryotic hosts. The gene self-assembly method of the instant invention provides an

alternative to *in vitro* recombination method of gene construction by permitting large constructs to be constructed.

One advantage of introducing the assembled product of this invention into a helper cell line to produce recombinant virus for the introduction of a gene or nucleic acid complex into a cell is that the assembled product will be auto-selected by the cells during the packaging process. Therefore, even where the overhanging termini have palindromic sequences, where there is more than one (but preferably less than four) unique complementary matches for a particular overhanging termini, or where concatamers have formed, only the correct or functional assembled products are expressed, transmitted, and assembled into virus. When the virus is then introduced into cells, the use of a reporter gene or another selectable marker provides yet a second layer of security for the selection of cells containing a properly assembled construct. For example, where a retrovirus helper cell line is used to produce a recombinant retrovirus containing the product of this invention (for retrovirus, RNA transcribed from the DNA product of the invention becomes packaged into the virus particle), a retrovirus-derived vector is transcribed as RNA and transmitted by packaging the RNA in a retrovirus particle. In order to be properly transmitted as a virus, the construct must be: 1) transcribed as RNA in a vector producer cell; 2) packaged into viral particles; 3) reverse transcribed into double-stranded DNA (in the recipient cell); and 4) integrated into the host chromosome. Each of these steps requires specific *cis*-acting sequences that must be correctly positioned within the vector. Thus, passage via retrovirus (or by other virus) is a means of auto-selection for the essential sequences.

In one application of the methods of this invention, the methods are used to rescue expressed sequences from RNA, or genomic sequences from cell DNA without disrupting the promoter sequences. Cellular transcriptional promoters are typically difficult to identify and isolate because they are generally not included in the RNA molecule and often extend over a considerable distance in a chromosome. One application of this invention relates to a promoter rescue technique that permits the entire promoter, or a fragment of a promoter to be isolated and cloned directly in to an expression vector without disruption of the flanking sequences. Promoter rescue techniques are known and include WO 94/20608 to Hodgson.

In a preferred embodiment of the invention, transcriptional promoters are cloned in a transcriptionally active manner for the selection and identification of new and/or

of tissue or cell-specific promoters enabling them to be used, selected, or screened for activity directly. For example, Fig. 3 illustrates one example of the formation of a vector for the incorporation of promoter sequences and the ultimate identification of those sequences using an exemplary plasmid VLBPGN (SEQ ID NO:1) as provided in Example 3, with *Bpm1* sites located within the locus of a retrotransposon (VL30) long terminal repeat (LTR). These methods preserve the structure and functionality of transcription factor response elements. The characteristic secondary structure of the LTR RNA remains very similar to the original LTR from which the promoter was rescued, thus preserving the important features of the original RNA/DNA molecule. Those of ordinary skill in the art will recognize that any of a variety of primers can be used with a variety of vectors and that the constructs of Figs 2 and 3 are exemplary and not limiting.

Fig. 2 illustrates the primers used to amplify the promoter insert (identified at a and c in Fig.2), and the insert region of the LTR (boxed), both of which can be digested at the same nucleotide position with *Bpm1*, to ensure a proper and seamless fit. In this example, after digestion of the vector, the two *Bpm1* sites leave non-complementary ends (a 3'-CC overhang on one end, and a 3'-GC overhang on the other). Thus, the ends will not efficiently anneal or ligate to one another. However, the complementary termini of the insert serves as linkage, enabling the plasmid to be completed by ligation.

In the example illustrated in Fig. 2, the terminus on the 3'-side (GC) is palindromic. Palindromic termini are self-complementary and can therefore ligate to themselves or to an identical terminus facing the opposite way (forming concatamers in the opposite direction). Despite the presence of palindromic termini and despite the potential for reduced fidelity in the self-assembling process, a large percentage of clones obtained by inserting promoter sequences into VLBPGN were assembled correctly (20/23). These levels are reduced somewhat when three or more fragments are combined for self-assembly, according to this invention and preferably, the use of palindromic termini are avoided when even numbers of nucleotides are exposed as overhanging termini because with even numbers of nucleotides there is an axis of symmetry. As noted above, where five base overhangs are used there are 1024 possible combinations of five nucleotides  $[(4)^5]$ , yet none of them is palindromic.

The vector of Fig. 3 is an example of a particular type of vector that is known as a retrotransposon vector. Retrotransposon vectors are described and reviewed in Hodgson



et al., 1996 *Retro-Vectors for Human Gene Therapy*. RG Landes Company, Austin TX, chapter 5 and see US Patent 5,354,674 to Hodgson. This type of vector is derived from a mouse cellular retro-transposon element that has no essential viral or cellular genes, and that has little sequence similarity to a retrovirus. However, this RNA (known as VL30 [virus-like, 5 30S]) has all the necessary *cis*-acting structural elements (such as LTRs and primer binding sites) required for efficient transmission by a type C murine or primate retrovirus. Thus, it is a parasite transmitted by retroviruses that is also expressed as a cellular RNA in most mouse cells and tissues. This RNA becomes packaged into retroviral particles when the mouse cells become infected by retrovirus. The retrovirus then transmits the VL30 (or a VL30 vector) to 10 the next infected cell (which can be a human cell). The RNA is then reverse transcribed and integrated into the DNA of the host cell.

Some advantages of VL30 vectors (over retrovirus-derived vectors) are: 1) lack of viral genes and other sequence homology that could lead to replication competent retrovirus (RCR); 2) ability to be expressed long-term *in vivo*; 3) a variety of LTR 15 transcriptional promoters that can be expressed in various tissues and under the influence of various hormones and other stimuli; and 4) the ability to express genes in a number of cell types that are targets of gene therapy. An additional advantage is that VL30 parts can be switched with those of classical retrovirus-derived vectors. For example, the LTR or packaging signal of VL30 can be used in place of the equivalent retroviral signal. The ability 20 to make mixed, or chimeric retro-vectors is a special application of gene self assembly technology.

Using a specific primer set, such as that shown in Fig. 2, or others, as taught in this invention, it is possible to amplify the U3 sequences expressed in the RNA of many different types of mouse cells. This is done using standard RNA isolation methods (Ausubel 25 *et al., supra*), coupled with extensive digestion with ribonuclease-free deoxyribonuclease, to eliminate residual DNA. Thus, to obtain a promoter that is expressed in the liver, one isolates RNA from liver and uses an RT-PCR procedure, such as those known in the art, with the primers to amplify the desired promoters. Fig. 6 illustrates liver RNA-derived promoters obtained using the methods of this invention. However, the promoters can also be derived by 30 conventional PCR from cDNA libraries (Fig. 5 illustrates T cell-derived promoters that were obtained in this manner). It is also possible to use the well-known hormonal and pharmacological inducibility of VL30 LTRs to find LTRs that are responsive to peptides,

hormones, and cytokines (for a table and description of VL30 pharmacologic responses (see Hodgson et al., 1996 *Retro-Vectors for Human Gene Therapy*. RG Landes Company, Austin TX, chapter 4, and Fig. 4.2). Examples of substances inducing various VL30 promoters to

high levels include: epidermal growth factor, basic fibroblast growth factor, insulin,

erythropoietin, glucocorticoid hormones, activators of cyclic 3'-5' AMP, and others. To

rescue promoters with pharmacological responsiveness, cells or animals stimulated with the

desired pharmacological agent are subjected to the RT-PCR procedure and the resulting U3

regions are cloned into a vector, (such as the exemplary VLBPGN) and are tested for

inducibility. Standard RNA blotting procedures can be used before isolating VL30

promoters, to determine whether a particular drug or hormone causes induction of VL30

RNA expression in a particular mouse cell or tissue. After the promoter has been rescued, the

vector is transmitted via retrovirus to the target cell (possibly a human equivalent of the

mouse cell from which the promoter was rescued). After selection with the drug G418 (400-

700 µg/ml, for 7-10 days) to select against cells not containing the vector, the target cell

population is challenged with the pharmacological agent of choice. Reporter gene expression

(in the example, GFP) or RNA expression, as determined by RNA blotting, can be used as an

assay of gene inducibility by the agent (for exemplary gene expression methods, see

Chakraborty *et al.*, *Biochem. Biophys Res. Commun.* 209:677-683, 1995).

Using any specific primer set designed for use with VL30 retro-elements and

using total cellular RNA from a particular mouse cell type as a template for RT-PCR, (using

commercially available kits and methods therein) candidate promoter elements can be

amplified. This method is useful for the identification of mouse-derived promoters and in

particular the method is useful for the identification of cell-type specific or tissue-specific

promoters from a mouse and for the selection of these promoters and the identification of

tissue-specific or cell-specific promoters that function in human cells. Thus, these types of

vectors and the methods for using these vectors permits the identification of promoters to

permit controlled transcription of a foreign gene. The promoters, originally obtained from the

mouse, can be used to effect tissue-specific or cell-specific expression in a human or animal

liver cell such as a hepatocyte, or in a human blood cell such as a T-helper cell or in an

erythrocyte (red blood cell). Methods are disclosed in Example 2 for the screening and

selection of the promoters from a library of amplified promoter sequences. Other methods

are well known to those of ordinary skill in the art. The specificity of the selected promoter

can be assessed, for example, by introducing a selectable marker under the control of the test promoter in question and introducing this construct into various cells to assess the ability of the promoter to selectively regulate expression.

The amplified fragments represent U3 promoter regions from any RNA species expressed in the originating cells and their abundance will be in approximate proportion to the number of expressed copies of RNA in the original mixture. Example 3 illustrates one example using a mouse T-helper cell cDNA library to produce amplified fragments representing U3 regions expressed in T cells. The vectors were efficiently expressed as RNA and protein in PA317 helper cells, and were transmitted by retrovirus into human T-helper cells, where they were integrated and expressed as protein in the form of a  $\beta$ -galactosidase reporter gene, as visualized by X-gal staining. The products of this experiment are provided in Fig. 5 and as SEQ ID NOS: 2 and 3 from T-helper RNA. The products of another experiment are shown in Fig. 6 as SEQ ID NOS: 4-13 from mouse liver RNA (by RT-PCR).

Examination of the different U3 sequences isolated from T cells and from liver revealed several things. First, the T cell U3 sequences were related to each other, as were the liver sequences. However, the two types of U3 sequences were quite different between the two sources (T-cell, Figure 5 and liver, Figure 6). Specifically, the liver sequences (Figure 6) appeared to be a closely related group, differing mostly by single point mutations, some of which may affect transcription factor binding sites. Some of the polymorphic sites included: a phorbol ester response element (VLTRE); a Rel/NF $\kappa$ b binding region, and a possible glucocorticoid response element (GRE). Some of these polymorphisms are illustrated in Fig. 6. The T cell-derived sequences (Fig. 5, SEQ ID NO:2 and 3), on the other hand, differed significantly in length, with SEQ ID NO:3 missing more than 120 bases (compared with SEQ ID NO:2) including putative binding sites for retinoids (RAR/RXR) and several elements contained within the enhancer repeat region (including a cAMP response element (VLCRE, or CREB/jun binding site), and putative serum response element (SRE, CARG, and NF1/IL6). SEQ ID NO:3 represented one out of five clones sequenced, while SEQ ID NO:2 represented four out of five. Possible sites of interactions between transcription factors and DNA can be observed by comparing the experimentally derived U3 sequences with those in Hodgson et al. (Retro-Vectors for Human Gene Therapy, 1996 Fig. 4.2 *supra*). In addition

to the deleted sequences of SEQ ID NO:2, there are a number of single base differences within the conserved regions of the two T cell-derived sequences.

Advantageously, a number of new VL30 promoter sequences (SEQ ID NOS: 2-13, *supra*) were identified using these methods despite the fact that VL30 RNA comprises only about 0.3% of cell mRNA represented in a cDNA library. Moreover, in each case, the cloned insert was isolated without the need to use linkers, adapters, or multiple cloning sequences such as those that are typically use for other library construction methods. The promoter sequences can be used in the vectors disclosed here to express inserted foreign genes or the promoter sequences can be substituted into other retroviral vectors, such as MoMLV-derived vectors or other VL30-derived vectors. Further, vectors containing the promoter sequences can be propagated in retroviral helper cells, such as PA317 (U.S. Patent 4,861,719 to Miller) or introduced into cells by chemical or physical transfection.

In another application of the methods of this invention, libraries of amplified sequences can be incorporated into vectors using two or more fragments and using the restriction endonucleases cleaving at a distance from their recognition sites. Preferably the vectors are created using six or more fragments and preferably greater than 10 or more fragments. For example, as applied to VL30 promoter sequences, because there are over a hundred VL30 retro-elements in the mouse genome, it is possible to amplify all of the promoter sequences *en masse*, and propagate them *en masse*, enabling screening by serial passage through helper cells (such as the PA317 helper cell line) or by means of a replication competent retrovirus, as illustrated in Examples 3 and 4. Conversely, the promoter region may be broken down into several sub-domains and permutations of each could be combined and screened to enhance the chances of generating a superior construct (Fig. 4B).

As an example of breaking a promoter region down into several sub-domains, Fig. 7 illustrates a similarity plot of nucleotide sequences found in VL30 U3 regions. Plot similarity was performed using the Plot Similarity program (Wisconsin Sequence Analysis Package, release 8.1, Genetics Computer Group, Madison, WI). This program plots the running average of the similarity among the sequences in a multiple sequence alignment. The sequences compared were those found in Fig. 4.2 of Hodgson, 1996, chapter 4 (*infra*). That is, the plot discloses the degree of conservation of VL30 promoter sequences among known VL30 promoters. From the figure, it can be seen that conserved sequences (close to 100% conserved) can be used as primer binding sites to amplify the adjacent sequences by PCR.

An allelic mixture of three fragment sets is then created to make a combinatorial library of promoters that can be positively selected, such as by using retroviral amplification of the active sequences. This, used in combination with the Fig. 4.2 (Hodgson, 1996, chapter 4 *supra*) can be used to determine regions of high similarity. Regions of high similarity within the U3 region can be replaced with one another. Therefore, a library of permutations of these sections can be made by combining allelic pools obtained by amplifying the sequences from individual subsections, followed by ligating the subsections in the correct order using the methods of the instant invention for gene self-assembly. For example, sub-section 1 can include the distal enhancer (from the LTR 5'-end to the site of insert primer 2, see for example the region defined by the insert primers 1 and 2 (SEQ ID NOS 55 and 56 of Example 4). In this way, using a plot similarity (such as Fig. 7), within each sub-section, the primers position fragments within a region of nearly 100% identity. Degenerate primers can also be used in these experiments to account for multiple nucleic acid base combinations along a particular sequence. In each case, the primers preferably are designed to have a melting temperature that is compatible with the RT-PCR conditions being used, and the conditions should be those recommended by the manufacturer (preferably Perkin Elmer Corp., Emeryville, CA). In Example 4, a set of primers is given that can be used to amplify different U3 subsections, together with directions for assembling a combinatorial library.

It will be appreciated by persons of ordinary skill in the art that the methods of the instant invention can thus be used to make allelic libraries of a variety of genes. For example, different allelic portions of a gene can be combined in a predetermined order and orientation to produce combinatorial libraries, without the need for fortuitous restriction sites separating the parts in the original construct, and without perturbing the important sequences joining the parts using the methods of this invention.

In this invention primers are constructed as described above. However, for the generation of allelic libraries or more complex library constructs it may be helpful to include 5'tags into the 5' end of the primer. The purposes of the tag sequence are: 1) to provide extra nucleotides on both sides of the restriction endonuclease recognition sites (for more efficient digestion); and 2) to enable recovery of sequence tags or undigested fragments by means of an affinity reagent (such as silica, magnetic beads, or nitro-cellulose containing the complementary sequences) for purification. The use of an affinity reagent permits the digested ends to be purified away from the digested fragments. Furthermore, if any

undigested ends remain after thorough digestion, the affinity reagent will remove them, further aiding in the purification. In one embodiment, affinity purification of the digested fragments is used in place of gel isolation, eliminating possible damage caused by ultraviolet light as well as possible damage caused by dye (e.g., ethidium bromide) binding to the DNA.

5 It will also be appreciated that a number of other variations to the primer sequences can be employed. For example, as discussed above, the enzyme recognition site for an enzyme that digests outside of its recognition sequence is included in the primer, so that the DNA digest creates an overlapping end that is complementary to one other terminus to which it will be joined. The enzyme recognition site can be moved to any location within  
10 the primer so as to digest the DNA at the exact location desired. The primer can also be programmed with a novel enzyme recognition sequence to add any desired sequences between the two sequences to be joined or to incorporate a linker or adapter if desired. If the sequences to be amplified contain the enzyme recognition site of the primers, it may be necessary to switch to a different enzyme usage. The use of several different enzymes is  
15 possible and has been discussed above. As with other PCR procedures, after the initial primer selections have been made the primers are assessed for their ability to fold back on themselves or to create internal secondary structure. The primers are preferably modified to avoid palindromic sequences or the potential for self folding within a primer. Nucleic acid analytical software (such as the Wisconsin GCG package, Oxford Biomolecular, Oxford, UK)  
20 is available to perform this analysis and aid in the selection of alternative primers.

In addition, as with all PCR processes, it is necessary to determine the melting temperatures ( $T_m$ ), and to adjust the annealing temperature of the PCR reactions to compensate for such temperatures. Finally, it is important to perform a sequence redundancy search, to determine whether the target sequence (the sequence complementary to the primer)  
25 is found more than once in the region to be amplified. If the sequence is repeated, it will be necessary to use a different primer in order to establish the single, correct priming site. Preferably, no more than 6-8 bases of incorrect target complementarity at the 3'-end of the complementary region is used and to allow a difference of at least 10° C between the  $T_m$ s of the correct and the incorrect target. The annealing temperature should always be at least 5°C  
30 lower than the  $T_m$  of the correct target and 5°C above the  $T_m$  of the incorrect target. Again, the necessary software and instructions are readily available from the cited sources (Wisconsin Gene Computer Group and Oxford Biomolecular, *supra*)

Next, a vector is constructed to include the appropriate elements for expression in the desired cell type. For example, the plasmid of Fig. 3A can be used for the creation of a promoter library or a vector can be created using a commercially available vector and primers to create a three or more fragment annealing and ligation reaction as provided above.

- 5 Preferably, the inclusion of a dominant negative selectable marker on the vector (*e.g.*, the neomycin phosphotransferase gene, conferring G418 drug resistance) can be used to reduce the likelihood that cells without the vector are being maintained in culture.

Multiple allelic copies of DNA (cell derived or cDNA) can be amplified in separate reactions as a set of potential inserts with each set having its own unique overlap  
10 sequence following digestion with a restriction endonuclease, according to this invention. The fragments can then be ligated into an existing vector or in a single reaction of three or more fragments to form a combinatorial collection of potential alleles. For example, if six adjacent regions are amplified from five separate alleles, the number of combinations would be  $5^6$ , or 15,625 potential combinations. The combinations can then be grown *en masse*, and  
15 selected *in vitro* or *in vivo*. A variety of screening strategies can be used in this invention and those of ordinary skill in the art will appreciate that the type of screen will match the type of library being generation. Therefore, for the promoter library, introducing members of the library into particular cell types to assess for expression in one or more cell types versus the absence of expression in another cell type is evidence of tissue-specific or cell-specific  
20 expression. For screening purposes, the libraries of this invention function like other libraries created through other methods. A variety of screening methods for a variety of libraries have been described in the art. For example, selective screens are reviewed by Hodgson et al. (1996, RG Landes Company, *supra*). Reporter protein production is well known in the art as is dominant selectable marker (*e.g.* drug) selection and selection by fluorescence activated  
25 cell sorting, antibody affinity selection, phage display selection (such as commercially available from Amersham, Milwaukee, WI), and the like can be used without detracting from this invention.

In this way, it is possible to isolate multiple forms of genes, gene fragments or regulatory regions such as transcriptional promoters or packaging signals (for example, in a  
30 retro-vector system). The individual constructs may then be tested *in vitro* or *in vivo* to further characterize a particular phenotype.

In one example the method is used to create a library of complementarity determining regions (e.g., allelic variations that give rise to antibody diversity) of antibodies or from receptors, including T-cell receptors, epitopes, antigens, ligands and the like. For example, where a library of T-cell receptors is created, the introduction of a vector designed to create a functioning T-cell receptor can be introduced into T cells or T-cell progenitors and the cells can be tested for their ability to bind to a particular test ligand. The ligand-recognizing cells can then be isolated from the ligand and grown in the presence of cytokines to produce specialized T cell clones. Where a library of antibodies or antibody fragments is created, the antigen reactive portions, for example, can be recombined in a vector containing the remaining portions of an antibody molecule to generate antibodies or antibody fragments in a cell. In other examples, the methods of this invention can be used to create allelic domains of receptor families (such as the steroid receptor super-family); libraries with related regions from peptide hormones; cytochromes P450; or other protein families that have shared domains or sub-sections with similar structures. The methods of the instant invention allow the joining of allelic sub-sections in an ordered fashion. In each case, it will be necessary to design primers, and to keep track of the uniqueness of joining overlaps and the presence of internal restriction sites as described above. While these will be different in each case, here are listed some general guidelines that are incorporated into the method of the instant invention.

As discussed above, although described as it relates to promoter libraries, libraries of other nucleic acid sequences can be created using the methods of this invention. These libraries include, introns and/or exons and/or functional domains libraries, libraries of potential alleles for a particular gene sequence, and the like. These sequences can be amplified from cell DNA or RNA using the primers of this invention and incorporated into a variety of vectors. For example, one vector of this invention, VLBPGN, has a portion of LTR removed and can be used to create a variety of libraries following digestion with *Bpm*1.

Selected or screened products of the combinatorial library can be used for gene expression, such as the promoters of Figs. 5 and 6. In addition, the exploitation of these sequences for the expression of a variety of genes, the LTR fragment containing the promoter can be joined to one or more functional retroviral packaging signals, internal ribosome entry sites, additional promoters, coding regions, processing sites, and the like.



Advantageously, there are almost no spatial constraints upon the joining of molecules by the method of the instant invention and other methods have not taken advantage of the combination of PCR to isolate genes or gene fragments; enzymes cleaving at a site distant from their restriction endonuclease recognition site to combine three or more fragments with precision; and, the use of unique overlapping non-palindromic termini to ensure fidelity of multi-fragment ligations. This combination permits the artisan to prepare complex gene constructions in one ligation step and does not require sequential sub-cloning into a vector or propagation in a prokaryotic host. Added to this the combination by these methods of fragment pools facilitates recombinatorial genetics.

The ability to recombine (in the correct order and direction) and screen a large number of allelic variants (whether as a simple library or as a combinatorial library), resulting in increased abundance (by amplification in the RNA, and subsequently in the DNA) is a special characteristic of this invention. Particular advantages of this system are obtained when the methods of this invention are combined with retrovirus vector technology or other virus vector technology. For example, the combination provides a form of *in vitro* evolution whereby the passage of the library through virus and through cells selects functioning sequences and increases the abundance of the surviving RNA and DNA molecules.

For example, consider the consequences of screening several different promoters expressing RNA in a donor cell (*i.e.*, a cell producing virus particles), but at differing levels of RNA abundance. In the following example, the least abundant RNA species is expressed at 0.1 copy of RNA per cell, while six others are expressed at 1 copy, 10 copies, 100 copies, 1,000 copies, or 10,000 copies, or 100,000 copies/cell, respectively. After a single passage, the DNA copy number in the recipient cells now reflects the approximate RNA copy number in the donor cells. These numbers are further amplified in the relative abundance of RNA species produced in the recipient cells. Disallowing for factors such as position effects, transcription factor depletion, etc., (which may be considerable), the same relative ratios of expression would be expected. Taking into consideration position effects, the disparity between abundance caused by changing insertion loci should average out. The most abundant RNA species after two passages is then many orders of magnitude more abundant than the least abundant.

Species:	RNA abundance: P=0	DNA copy no. P=1	RNA abun. P=1	DNA copy no. P=2	RNA abun. P=2
A	0.1 copy/cell	0.1	0.01	0.01	0.001
B	1	1	1	1	1
C	10	10	100	100	1,000
D	100	100	10,000	10,000	10 <sup>6</sup>
E	1,000	1,000	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>9</sup>
F	10,000	10,000	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>12</sup>
G	100,000	100,000	10 <sup>10</sup>	10 <sup>10</sup>	10 <sup>15</sup>

**Table 2.** Enhancement of DNA and RNA copy number as a result of different RNA expression levels, after retroviral passage. P= (no. of passages). Numbers are interpreted as relative ratios within a column.

5

The present invention is able to efficiently create a library of RNA or DNA sequences whether or not they are in low abundance. The kinetics of screening for RNA abundance of a promoter can be appreciated best in the following discussion. For the purposes of this discussion, position effects have been ignored. An equation describing the kinetics of screening for RNA abundance is:

10

$$(1) R_{\text{rel}\chi} = A_{\chi} / \sum A_{n \rightarrow \infty}$$

15

The above equation (1) can be stated in plain English: The relative abundance of an RNA species  $\chi$  ( $[R_{\text{rel}\chi}]$ ) within a population of RNA molecules expressed in a single cell or within a population of cells) is equal to the RNA copy number of RNA species  $\chi$  ( $A_{\chi}$ ) divided by the sum of the RNA copies of all RNA species present, including  $\chi$ .

The relative abundance number of any given species changes as the number of passages change, according to the following approximation:

20

$$(2) R_{\chi py} = D_{\chi p0} R^{p+1}$$

25

In the simplest of terms, equation two (2) can be expressed as: The abundance of RNA species  $\chi$  after Y passages ( $R_{\chi py}$ ) is equal to the initial abundance of the DNA for species  $\chi$  at passage=0 ( $D_{\chi p0}$ ), multiplied by the RNA abundance/DNA copy, raised to the power of the number of passages plus one. Thus, a typical RNA species that starts out as a

single copy of DNA, after zero passages (*i.e.*, in the donor cell) expresses 10 copies of RNA/cell. After one passage it is amplified at the DNA level to a relative ten copies (the same as the RNA abundance at  $P=0$ ), and at the RNA level to 100 copies (10 copies per DNA copy). The reason for the amplification is that viral packaging and passage is based upon the number of RNA copies present in the donor cell. These calculations can be used to arrive at approximate abundance determinations for any given passage. The actual results of any given experiment, of course, will be biological rather than physical or mathematical. This means that other variables such as RNA efficiency of transmission and longevity, availability of transcription factors, experimental variation, *etc.* also come into play. The underlying purpose of the approximating equations, however, is to illustrate that RNA is amplified in DNA in proportion to the abundance of the template (RNA) within the cell.

The abundance of mRNA in cells can vary continuously from less than a copy per cell to nearly 100,000 copies/cell in actively transcribing, highly-specialized cells such as reticulocytes, the chicken oviduct, the silk moth silk gland, *etc.* Therefore, the spectrum of RNA abundance from 0-10<sup>5</sup>/cell is within the biological window of interest. For most practical purposes, such as biotechnological expression of genes in specific cells, only the higher end of this abundance range is desired. Therefore, using a viral selection system, as disclosed in this invention, it may be possible to disregard those species with less than a threshold level, such as <0.1 copies per cell. The selection through virus will lead to the recovery of the more abundant species. Furthermore, because the vector is likely to be the only considered sequence, it may be considered as a proportion of the whole of RNAs expressed in the target cell. The situation is more complex when a large number of permutations and combinations is generated, for example by self-assembling thousands or millions of fragments in a predetermined order using the self-assembly technique of the instant invention. Consider the assembly of allelic variants of four promoter subregions: distal enhancer, proximal enhancer, distal promoter and proximal promoter. If 100 varieties of each of the four groups were amplified and combined using the instant process along with a single vector, 10<sup>8</sup> resultant combinations could occur. However, a sufficient number of molecules to start out a combinatorial screening program might be a million. The problem can be simplified by considering these in groups as follows:

**Table 3. Grouped abundance of RNA molecules derived from combinations.**

No. of species in group:	RNA abundance:	Total No. molec. at P=0:	RNA at P=1	RNA at P=2	RNA at P=3
$9 \times 10^5$	1	$9 \times 10^5$	$9 \times 10^5$	$9 \times 10^5$	$9 \times 10^5$
$2 \times 10^5$	10	$2 \times 10^6$	$2 \times 10^7$	$2 \times 10^8$	$2 \times 10^9$
$2 \times 10^4$	1,00	$2 \times 10^6$	$2 \times 10^8$	$2 \times 10^{10}$	$2 \times 10^{12}$
$1 \times 10^3$	1000	$1 \times 10^6$	$1 \times 10^9$	$2 \times 10^{12}$	$2 \times 10^{15}$
$1 \times 10^1$	10,000	$1 \times 10^5$	$1 \times 10^9$	$1 \times 10^{13}$	$1 \times 10^{17}$
1	100,000	$1 \times 10^5$	$1 \times 10^{10}$	$1 \times 10^{15}$	$1 \times 10^{20}$
<b>Sum Total:</b>		$6.6 \times 10^6$	$1.11 \times 10^{10}$	$1.01 \times 10^{15}$	$1 \times 10^{20}$

Thus, it follows that in the example population (Table 3) of over a million constructs (equally represented in the DNA), a single construct expressing  $10^5$  copies of RNA per DNA copy will increase to approximately 99% of the total expressed RNA sequences in two passages. Using similar procedures in combination with drug and/or hormonal stimulation, and after consideration of the possible transcription factor binding sites within the sequence family (Figs. 5 & 6), it is within the intended scope of the invention to select for hormonal or pharmacological controls of transcription such as have been described herein. The factors contributing to the outcome are not only the input constructs, but recombinants and mutants as well. These secondary contributors to molecular diversity will be enhanced if multiple rounds of infections are allowed to occur, as oftentimes the difference between a particular transcription factor being able to bind (or not) may depend upon a single base change. Because viral infection is progressive and competitive, molecular evolution can be used to generate gene constructs *de novo* in the tissue culture dish in short time periods. Advantageously, the use primers to generate amplified fragments with uniquely complementary cohesive ends (i.e., that the ends will preferably only hybridize with the intended 5' and 3' fragments) to ligate three or more fragments as taught in this invention improves the potential for obtaining a diverse library.

Although the examples particularly point out a transcriptional promoter as the product of the process, the skilled artisan can appreciate that a particular selection technique can be applied to other *cis*- and *trans*-acting genetic sequences as well. Although a virus is used to propagate the selective advantage of a preferred embodiment, it can also be appreciated that any selective screen, such as drug selection, cell survival, phenotypic selection, cell sorting, antibody selection, and the like (see Ausuble et al., *supra*) could be

substituted without changing the intended scope of the invention. Likewise, transfection or cell fusion could be used in place of viral infection. Furthermore, substitution of different viruses, retrotransposons, or functional groups are likewise within the intended scope of the invention. The described embodiments are to be considered only as illustrative and not restrictive, and the scope of the invention is indicated by the claims rather than by the narrative description. All references and publications, cited herein, are incorporated by reference into this disclosure.

Like the embodiments detailed above, the method of library production is also conducive to assembly and transfer of genetic material directly into eukaryotic cells, saving the step of propagation in bacteria that is standard in bacteria. An advantage of direct transfer of the libraries of this invention to eukaryotic cells, including the exemplary retroviral vector producer cells, is that certain essential *cis*-acting structural features will be under positive selection (i.e., if they are not present, the molecule will be lost due to its non-functionality). As discussed above, it is often advantageous to eliminate bacterial and plasmid DNA sequences, endotoxin, and other bacterial contaminants by introducing the constructs directly into eukaryotic cells.

In addition to providing a method for constructing complex DNA molecules efficiently (as in the examples of three piece and six piece constructs), the methods of this invention permit the assembly of constructs that are larger than those conventionally propagated in *E. coli*. Examples of these types of vectors include adenovirus vectors, herpes simplex vectors and artificial minichromosomes. In order to insert genes into such vectors that are too large for conventional molecular cloning procedures, in the past it was often necessary to resort to *in vivo* recombination, wherein the genes of interest are cloned into a suitable vector and the flanking homologous regions are used to target the foreign genes to a homologous site within the larger viral or minichromosome vector. However, the methods of this invention permit PCR fragments of any size (up to the limits of PCR capability, 20-30 kb per fragment) to be joined together. Thus, it is feasible to precisely construct adenovirus vectors by amplifying larger sequences, and combining them by ligation. For example, several sections of adenovirus (5-10 kb each) can be ligated using the methods of this invention, up to for example, about 37 kb, and then transformed directly into human cells. Only the correctly recombined vectors are capable of replicating. Hence, the DNA is autoselecting. A similar procedure is used for generating herpes virus vectors, which are

approximately 150 kb. The precision of the methods of this invention permit non-essential viral genes to be more easily eliminated from the construct. After transfection into appropriate cells, the DNA replicates and virus particles are formed.

Some special considerations apply to larger vectors, however. First, it is  
5 desirable to use enzymes that do not cut within the large DNA fragments. To prevent excessive fragmentation of the DNA by internal sites, it is desirable to use enzymes that cut rarely or infrequently, such as CpG-containing enzymes recognizing six bases, or enzymes such as *Sap1*, recognizing seven bases and digesting a three bp overhang (thus permitting up to 32 fragments to be joined in order). It is also desirable to avoid shearing the DNA once  
10 large segments have been joined by ligation. One method of avoiding shear is to add the transfection agent, such as Superfect<sup>™</sup> reagent (dendrimers, Qiagen) or Lipofectamine<sup>™</sup> (liposomes, Life Technologies, Gaithersburg, MD) directly to the ligation reaction, and then add the cells to be transfected to the mixture. This, or a similar method avoids the need to physically move the ligated DNA, and thus prevents shearing. Another method is to add a  
15 DNA condensing reagent (dendrimers, polycations [such as polyethylenamine] histones or liposomes) directly to the DNA ligation reaction, and then move the DNA by pipette after it has condensed (thus reducing shearing of the DNA). Once inside the cell, viral DNA can replicate (as in the examples of partially replication-competent adenovirus and herpes simplex virus vectors).

20 Artificial minichromosomes have been under development for years. True artificial chromosomes require a centromere, at least one origin of DNA replication, and in the case of linear molecules, telomeric repeats at the chromosomal termini. In addition, to be very effective it is desirable to have a selectable marker gene, one or more therapeutic genes, and/or reporter genes.

25 In reality, the use of minichromosomes has been delayed by the inability to effectively manipulate the larger DNA molecules *in vitro*. Yeast and bacterial artificial chromosomes have been used with little success in mammals, and the addition of telomeres to the ends of linear chromosomes is also a special problem, as there is no prokaryotic host that can tolerate large linear DNA. The methods of this invention offers the opportunity to  
30 assemble human or mammalian minichromosomes *in vitro*, by using large segments (10-30 kb) of synthetic, gene-amplified DNA as ligation starting materials. For example, up to 32 *Sap1* fragments (up to 30 kb each, containing the essential *cis*- and *trans*-acting sequences),

or 512 shorter *Hga*1 fragments can be combined using these methods. As with the other examples, several enzymes suitable for this invention (e.g., such as class IIS enzymes) can be combined (possibly with different termini lengths) to simplify the task. The methods of this invention also facilitate construction of telomeric repeats, because the constructs of this invention do not need to be circular. Thus, the methods of this invention can be used to make telomeres of any length, by adding additional segments onto the ends of molecules. One way to do this is using self assembling genes that employ a repeating overhang sequence (self-complementary molecule, such as AG-3' at one end, and CT-3' at the other end), permitting the telomeres to be lengthened to the extent desired by adding the required molar excess of the telomeric repeat-containing fragment. This technique gives the investigator some control over the relative length of the telomeres, although the self-complementarity indicates that many repeats will be lost due to self-ligation. This can be alleviated by using higher starting concentrations of DNA to favor inter-molecular ligations over intra-molecular ligations (e.g., >20 µg/ml starting concentration of DNA).

A two fold molar excess of telomeric fragments gives approximately twice the average length of telomere as a strictly 1:1 molar ratio of all fragments. By using a higher molar ratio of shorter telomeric repeats it is possible to give greater uniformity to the overall length of the molecules, which will vary from one terminus to the other. Thus, in addition to providing a way to build large molecules with precision, the methods of this invention provides for a way to control the telomere length (or potential life-span) of the artificial chromosome. To prevent damage during handling, the minichromosome DNA can be condensed with polycations, adenovirus particles, dendrimers, histones, or liposomes prior to transfection, similar to larger viral vectors.

The methods of this invention can be used to create recombinant virus. One example of this is an adenovirus vector self-assembling gene system. This system can include three parts: 1) vector; 2) helper virus; and 3) helper cells. The vector part is a self-assembling fragment set of at least three fragments comprising the essential cis-acting sequences (left and right inverted terminal repeats, which are the 103 bp at both ends of the genome that are required for replication [ITRs] and packaging sequences [Y, base pairs 194-358) and central 'baggage' area, comprising one or more self-assembling fragments including therapeutic genes, marker genes, and reporter genes. The baggage area is thus flanked by the cis-acting sequences in the vector. Because the synthetic oligonucleotide sequences

comprising the 5' and 3' termini of the helper virus are not phosphorylated, they will not ligate together creating multimers. Thus, the Ad5 vector region will assemble only into monomers. The helper virus part comprises all Ad5 trans-acting genes except for the E1A and E1B genes. The helper virus part has no cis-acting sequences, and it is amplified in several sections. In this preferred embodiment, the virus is amplified using primers that exclude the ITRs, packaging region and E1A&B genes. The helper virus is digested by *Sap1* digestion, creating seven uniquely terminated fragments comprising the trans-acting viral genome, with dephosphorylated, blunt 5' and 3' ends on the terminating fragments. The primers are designed so as to amplify the internal virus sequences without changing them, except for the 5' and 3' ends of the virus. The PCR-amplified fragments are digested with *Sap1* and are religated in their natural order after gel isolation and Qiagen column purification. The 5' end of the helper virus genome starts at 3.2 kb (in the E1A gene) so as not to overlap the vector sequences, which could otherwise cause replication competent adenovirus (RCA). Because the 5' and 3' ends of the helper virus do not contain *Sap1* sites, they remain intact after digestion with *Sap1*. Because the synthetic oligonucleotide sequences comprising the 5' and 3' termini of the helper virus are not phosphorylated, they will not ligate. Thus, the Ad5 helper virus genome assembles only into preferred monomers during ligation.

In a preferred embodiment, non-essential genes are deleted from the Ad5 genome by means of the method of self-assembling genes. In another preferred embodiment, the helper virus genome is approximately 30 kb after deletion of E1A, E1B and E3 gene sequences from the helper virus, and it is amplified as a single long fragment using the eLONGase Amplification System (Life Technologies or a similar strategy for creating long PCR fragments with high fidelity). It is not of great importance that occasional PCR errors may occur, because multiple copies of the Ad5 helper virus are transfected into target cells, thus providing trans-complementation. The helper cells are preferably 293 cells, a human kidney cell line expressing E1A and E1B genes (ATCC). The vector part and the helper virus part are combined in equimolar ratios after ligation has been performed separately on each fragment set. The Superfect protocol (Qiagen) is used to transfect the vector part and the helper part into the helper cells. The helper cells lyse, releasing high-titer adenovirus particles that are capable of infecting a variety of human cells. The resulting defective virus is incapable of forming RCA, and it transmits up to 34 kb of foreign genes in the baggage area. Unlike conventional Ad5 vectors that require separate constructs for *E. coli* propagation of



insert genes, and recombination in vivo, the present vectors are relatively easy to make and provide a precise, safe alternative to first generation and second generation adenovirus vectors.

Exemplary methods for producing self-assembling vectors and genes are provided below. Further, the Examples provide methods for producing libraries of nucleic acid sequences using the methods of this invention. A number of nucleic acid sequences identified using the methods of this invention are described. The examples provided below are exemplary and not limiting. All references and publications provided herein are incorporated by reference into this disclosure.

### Example 1

#### Three-Piece Gene Self-Assembly with 100% efficiency

Using 6 primers (SEQ ID NOS:24 and 63-67), three PCR fragments were amplified from templates VLMG (SEQ ID NO:22) and VLBPNG (SEQ ID NO: 1). PCR reactions were carried out using the hot start technique, according to the manufacturer's instructions (Perkin Elmer) using *Pfu* DNA polymerase (Stratagene). To amplify specific portions of the above templates, each primer contained a class IIS enzyme site capable of digesting a unique overhanging end that was complementary to only one other terminus in the subsequent ligation. The class IIS enzymes used were *Bpm1* and *Eco 57I* (the latter was used to copy a fragment that contained an internal *Bpm1* site). The reactions were carried out as follows: 1) the lower reaction was assembled according to the protocol for PCR Gems (Perkin Elmer); 2) the lower reaction was heated to 80°C, 5 min, then cooled to 4°C for 5 min; 3) the upper reaction was prepared according to PCR Gems protocol and was added to the lower reaction (separated by cooled wax). The primer concentration was 0.3 µM (final). The dNTP concentration was 200µM (final). 5 Units of *Pfu* polymerase was used. All fragments were amplified using the following conditions: 96°C, 45 sec; (then followed by 30 cycles of the following) 96°C 45 sec, 52°C 45 sec, 72°C, 6 min; then followed by a single incubation at 72°C for 10 min; then hold at 4°C. All fragments were successfully amplified. The PCR fragments were purified using the Qiaquick PCR purification protocol (Qiagen). The fragments were digested with an excess of the appropriate restriction enzyme (*Bpm1* or *Eco57I*). The digested fragments were run on a 1% agarose gel and were excised using minimal irradiation from a hand-held 365 nm ultraviolet light. The fragments were purified

using the Qiagen Qiaquick Gel Purification Protocol. The fragments were ligated at an equimolar ratio at a concentration of >20µg/ml with T4 DNA ligase (Boehringer Mannheim) overnight at 4°C. Competent *E. coli* SCS110 cells (Stratagene) were transformed with the ligated DNA. Eight colonies were characterized by restriction enzyme analysis, and all eight  
5 contained the correct order and orientation of the three fragments. The experiment was repeated independently by another investigator, and the same result was obtained (8/8=100%). Thus, the procedure resulted in a high percentage of correctly assembled vectors.

This three-piece vector was VLΔBP. The deletion extended from the distal  
10 enhancer region to the TATA box near the start of transcription. The deletion region was a pair of *Bpm1* sites that permitted U3 sequences to be cloned into the insert.

One validated *E. coli* clone of VLΔBP was transfected into retroviral helper cells. After 48 h, the vector was transduced into amphotropic helper cells. After selection for two weeks with the drug G418, drug resistant colonies were grown up in a mass culture and  
15 the vector was transduced from the amphotropic helper cells into a human HT1080 cell line (ATCC, Rockville, MD). Surprisingly, even with a large deletion in the LTR promoter, the basal TATA box-containing VLΔBP was transmitted as a retrovector and was permanently inserted into the human cell line, thus establishing the validity of the self-assembly technique for the construction of functional eukaryotic vectors.

20

## **Example 2**

### **Production of a Six Piece Self-Assembling Expression Vector**

Due to the high efficiency of the gene self assembly process for the three piece  
25 assembly, a complex vector containing six fragments was constructed. The results here were extended to determine whether such a self-assembled vector would also have biological activity in human cells without being cloned and grown in a prokaryotic cell.

Six fragments were individually constructed by PCR using three different templates and twelve primers (as illustrated in Fig.8). The primers used three different class  
30 IIS enzymes. The enzymes were chosen so as to give 2 base pair, 3'-overhanging ends. Three enzymes were used in order to avoid the use of enzymes that had additional sites internal to the fragments being amplified. Thus, *Bpm1* was used unless there was an internal *Bpm1* site. If such a site existed, *Eco57I* was used. If there was also an internal *Eco57I* site, then *BsrD1*

was used. However, it is alternatively possible to use an enzyme such as *Eam*11041, where the *Eam*11041 sites in the primers are unmethylated (therefore susceptible to digestion by the enzyme), and wherein the <sup>m</sup>5dCTP analog of dCTP is used in the PCR reaction, methylating all internal sites (and protecting them from digestion by *Eam*11041), as suggested by Padgett and Sorge, 1996, *supra*, and incorporated herein by reference.

Using 12 primers, 6 fragments were amplified from 3 templates: pBK-CMV (SEQ ID NO:26), pVLMB (SEQ ID NO:23) and pVLOVhGH-900 (SEQ ID NO:21). Fragment 1 was amplified from pBK-CMV using primers 1 and 2 (SEQ ID NOS:31 and 32). Fragment 2 was amplified from pVLMB using primers 3 and 4 (SEQ ID NOS:33 and 34). Fragment 3 was amplified from pVLOVhGH-900 using primers 5 and 6 (SEQ ID NOS:35 and 36). Fragment 4 was amplified from pVLMB using primers 7 and 8 (SEQ ID NOS:37 and 38). Fragment 5 was amplified from pVLMB using primers 9 and 10 (SEQ ID NOS:39 and 40). Fragment 6 was amplified from pVLMB using primers 11 and 12 (SEQ ID NOS:41 and 42). PCR reactions were carried out using the hot start technique, according to the manufacturer's instructions (Perkin Elmer Ampliwax PCR GEMS 100). The lower reaction was heated to 80 °C for 5 min, then cooled to 20 °C for 5 min. The upper reaction was prepared according to PCR gems protocol and was added to the lower reaction (separated by cooled wax). The primer concentration was 0.3 micromolar (final). The dNTP concentration was 200 µM (final). 5 U of *Pfu* polymerase (Stratagene) was used per reaction. 100 ng of template was used for each reaction. 14 rounds of PCR amplification were used to reduce mutagenesis of the templates. The PCR cycling protocol was 96 °C 45 sec; then two cycles of (96°C 45 sec, 52°C 45 sec, 72°C 6 min); then 12 cycles of (96°C 45 sec, 58°C 45 sec, 72°C 6 min) followed by a 72° C soak for 10 min, then to 4°C hold.

The six PCR fragments were designed to self-assemble into a retro-vector after digestion with the correct class IIS restriction enzyme (Fig. 8). After transfection into retroviral helper cells, the vector DNA is transcribed as RNA by means of the cytomegalovirus immediate early promoter (fragment 1). This promoter replaces the retroviral or VL30 LTR in this vector. The RNA transcript region begins with the R and U5 regions of the Moloney murine leukemia virus (MoMLV) LTR, the viral packaging signals (Ψ) region of MoMLV, the packaging enhancer (Ψ+) region of mouse VL30 and the IRES region of EMCV fragment 2. Fragment 3 consists of the human growth hormone (hGH) cDNA sequence. Fragment 4 consists of the SV40 virus early region promoter driving

expression of the neomycin phosphotransferase (neo) gene. Fragment five consists of the (+)-strand primer binding site of the MoMLV LTR, the U3 region of the MoMLV LTR, the repeat (or R) region, and a portion of the U5 region. Fragment 6 consists of the PBR322 plasmid origin of replication.

5

Fragment 1: CMV early region promoter

Template: pBK-CMV plasmid DNA (Stratagene, LaJolla, CA) *Bpm1* (SEQ ID NO:26)

PCR primer 1 (SEQ ID NO:31)

10 GACTAACCTTGATTCCACTGGAGCCGTATTACCGCCATGCATTAGTTATTAATAG

PCR primer 2 (SEQ ID NO:32)

GACTAACCTTGATTCCACTGGAGTAATTGCGGCTAGCGGATCTGACG

Fragment 2: R-U5-Psi-Psi(+)-IRES *Bpm1*

15 Template: pVLMB plasmid DNA (SEQ ID NO:23)

PCR primer 3: SEQ ID NO:33

GACTAACCTTGATTCCACTGGAGACACTTGACCTCTACCGCGCCAGTCCTCCGAT  
TGACTGAGTCG

PCR primer 4: SEQ ID NO:34

20 GACTAACCTTGATTCCACTGGAGGGATCCGCGCCCATGATTATTATCG

Fragment 3: human growth hormone (hGH) *Bsr* DI

Template: pVLCNOVhGH plasmid DNA (SEQ ID NO:21)

PCR primer 5: SEQ ID NO:35

GACTAACCTTGATTCCAGCAATGTCGGTTAGCTTGTTTCTTTACTGTTTGTC

25 PCR primer 6: SEQ ID NO:36

GACTAACCTTGATTCCAGCAATGTTAGGACAAGGCTGGTGGGCACTGG

Fragment 4: SV40 early promoter-neomycin phosphotransferase

Template: VLMB plasmid (SEQ ID NO:23)

30 PCR primer 7: SEQ ID NO:37

GACTAACCTTGATTCCACTGGAGGGTCGACCCTGTGGAATGTGTGTCAG

PCR primer 8: SEQ ID NO:38

GACTAACCTTGATTCCACTGGAGAATCTCGTGATGGCAGGTTGGGCGT

Fragment 5: MLV(+)/PBS-U3-R-U5

5                   Template: VLMB plasmid (SEQ ID NO:23)

PCR primer 9: SEQ ID NO:39

GACTAACCTTGATTCCACTGAAGAGATTTTATTTAGTCTCCAGAAAAAGGGGGG

PCR primer 10: SEQ ID NO:40

GACTAACCTTGATTCCACTGAAGCCCCCAAATGAAAGACCCCCGCTGACG

10

Fragment 6: PBR322 origin of replication

Template: VLMB plasmid (SEQ ID NO:23)

PCR primer 11: SEQ ID NO:41

GACTAACCTTGATTCCACTGGAGCCGGGACGGAATTCGTAATCTGCTGC

15

PCR primer 12: SEQ ID NO:42

GACTAACCTTGATTCCACTGGAGTTCTCGAGGCGGCGCATCTCGGCG

*Procedure:* The twelve primers were prepared by the following procedure: 1)

oligonucleotides were synthesized with trityls off. After deprotection and lyophilization, the  
20 samples were resuspended in 5 microliters deionized formamide and loaded onto a  
polyacrylamide gel (12% polyacrylamide, 250V). The samples were excised under short  
wave UV irradiation and eluted overnight in 600 microliters of sample elution buffer (0.5 M  
ammonium acetate, 10 mM Mg acetate, 1 mM EDTA, 0.1% SDS). The contents were loaded  
onto a BioRad Chromatography column (Cat. # 732-6008) and centrifuged into an Eppendorf  
25 tube at low speed (2000 RPM, 5 min). After washing the column with 500 microliters TE  
buffer (10 mM Tris, 1 mM EDTA), pH 8.0 and recentrifugation (2000 RPM, 5 min), the  
pooled eluate was ethanol precipitated, washed with 100% ethanol, resuspended in TE buffer  
and quantitated by spectrophotometry of a small sample, which was then discarded.

Fragments were cleaned using the Qiaquick PCR cleanup procedure. The  
30 fragments were digested with their respective class IIS restriction enzyme. The digested  
fragments were run on 1% agarose gels, and the fragments were excised and cleaned using  
the Qiaquick gel cleanup procedure. Fragments were combined in an equimolar mixture and

ligated overnight at 4° C with T4 ligase and ATP. An analytical gel was run with the ligated DNA, as well as with controls including unligated fragments and ligated fragments with a single fragment missing. As opposed to the controls, the complete ligation included bands equivalent to the full-length supercoiled monomer (referred to as GENSA 981, SEQ ID NO:29), as well as bands possibly representing multimers (up to six bands were observed).

In order to assess the efficiency of the method, eleven nanograms of DNA were transfected into SCS1 supercompetent cells. Thirteen kanamycin resistant colonies were harvested, and plasmid DNA preps indicated 10 out of thirteen that appeared to be the correct length. All ten gave the expected bands when digested with *Pst*I, *Sna*BI, and *Bam*HI. 1.35 µg of the ligated DNA was purified by phenol-chloroform-isoamyl alcohol extraction, followed by two extractions with chloroform-isoamyl alcohol, and was precipitated in ethanol. The DNA was washed in 70% ethanol and re-suspended in 50 µl of sterile phosphate buffered saline (for transfection). The DNA was transfected (using the Qiagen Superfect protocol) into HTam1 (amphotropic human helper cells). 24 h after transfection, the target cells were washed and fresh culture media was added. 48 h after transfection, the supernatant from the vector producer cells was filtered (0.45 µm, Nalgene) and transferred to PG13 helper cells (ATCC) and HT1080 human fibrosarcoma cells. This procedure was repeated after 72 h. 48 h after transduction, recipient cells were started on G418 drug selection (500 µg/ml). The appearance of G418 drug-resistant colonies on transduced PG13 and HT 1080 cells after 6 days of selection indicated successful transmission via retrovirus particles. The transfect HTam cells were also selected with G418. After six days of drug treatment, 45 colonies of resistant cells were counted. Thus, the six fragment gene assembly was effectively transmitted and expressed as either a DNA (transfection) vector or a retro-vector.

### Example 3 Design and Construction of Single LTR Vectors

**Background:** In order to manipulate the interior of the VL30 LTR sequences using a promoter rescue technique, single LTR vectors were constructed. The mouse VL30 element NVL-3 was used as the starting material as it is constitutively and abundantly expressed in most mouse tissues. Single LTR vectors are circular and behave as if they contained two LTRs. Thus, in these vectors RNA transcription begins at the start of the R region (see Fig.

3B), and continues through the polyadenylation site after completing the second round of transcription of the R sequences (Fig. 3A). In previous studies, these vectors were expressed transiently in vector producer cells and the DNA did not integrate into cell DNA as a standard two LTR vector. Therefore, the vectors were usually passed to a second complementation helper cell line via retroviral transduction of the vector RNA transcribed in the first helper cell. This process resulted in the vector regenerating a correct (two LTR) structure upon integration into the recipient cell DNA.

*Experimental method:* The plasmid pNVL-3 (SEQ ID NO:25, kindly provided by Dr. J.

10 Nortonm Manchester, UK), containing a complete copy of the NVL-3 (mouse VL30) genome (Adams *et al.*, 1989), was digested with *Xho*I (which cuts in the LTRs), releasing the 4.27 kb VL30 genome with one copy of the LTR. This fragment was circularized using T4 DNA ligase and ATP. The circular DNA was linearized by digestion with *Sna*BI, 187 bp from the 3'-LTR. A 2.3 kb fragment containing the SV40 virus early region promoter and the  
15 aminoglycoside phosphotransferase (*neo*) gene, together with the PBR322 plasmid origin of replication, was excised from the BAG retrovirus vector (Price *et al.*, *Proc. Natl. Acad. Sci.* 84:156-160, 1987, kindly provided by C. Cepko, Cambridge, MA). BAG is also obtainable in a retrovirus helper cell line from American Type Culture Collection (ATCC), Rockville, MD by digestion with *Xho*I and *Bam*HI. This fragment was blunted with T4 DNA  
20 polymerase and dephosphorylated with calf intestinal alkaline phosphatase (CIP). The fragment was then ligated to the linearized *Sna*BI fragment of NVL-3. The resulting plasmid (containing a circularly permuted NVL-3 genome with the SV-*neo-ori* region) was designated VLSNO2 (SEQ ID NO:30).

In order to facilitate the switching of LTR sequences by means of the class IIS  
25 enzyme *Bpm*I, VLSNO2 was digested with *Bpm*I (six sites). The region containing four *Bpm*I sites was removed and replaced with a 19 bp linker (SEQ ID NOS: 1 and 52, see below), 921 bp beyond the LTR. The linker contained *Sna* BI, *Cla*I and *Bam* HI cloning sites.

Linker (top strand): 5'-TACGTATCGATGGATCCGA-3' (SEQ ID NO:51)

30 Linker (bottom strand): 5'-GGATCCATCGATACGTAAG-3' (SEQ ID NO:52)

The remaining two of the *Bpm1* sites had complementary ends, which permitted their ligation and resulted in eradication of all *Bpm1* sites within the resulting vector VLSNO3 (SEQ ID NO:20).

In order to facilitate reporter/therapeutic gene function, a 3.7 kb fragment containing the internal ribosome entry site (IRES) from encephalomyocarditis virus, together with the  $\beta$ -galactosidase reporter gene, was excised from the plasmid pVLSAIBAG (kindly provided by Mr. James Grunkemeyer, Omaha, NE) by means of a partial digestion of the plasmid with *Bam* HI. This region was inserted into the *Bam* HI site of VLSNO3, resulting in the vector VLSNOSIB (SEQ ID NO:14).

A second reporter construct, pVLSNOG (5774 bp, SEQ ID NO:19) contained the green fluorescent protein (GFP, Clontech, Palo Alto, CA) gene was constructed by inserting a *Bgl2-Bcl1* fragment (800 bp) from plasmid pGFP-N1. This sequence, containing the GFP gene, was treated with mung bean exonuclease and inserted into the unique *Sna* B1 site of pVLSNO3.

In order to enhance GFP fluorescence from the reporter plasmid pVLSNOG, the serine-65 codon in the GFP gene was mutated into threonine by a site-directed mutagenesis procedure with the Transformer™ Site-Directed Mutagenesis kit from Clontech. A *Bpm1* site in the GFP gene (threonine-9) was mutated at the same time without changing the amino acid (ACT to ACA). The resulting plasmid was pVLSNOGM (SEQ ID NO:18).

An *Nco1-Xho1* fragment (585 bp) from plasmid pG1IL2EN (kindly provided by Dr. Steven Rosenberg, Bethesda, MD), containing the internal ribosome entry site (IRES) from encephalomyocarditis virus (EMCV) was inserted into the *Apa1* site upstream of the GFP gene in pVLSNOGM, resulting in pVLSNOGMI (SEQ ID NO:17). Both insert and plasmid fragments were blunted with mung bean exonuclease. One variant version of pVLSNOGMI with an IRES tandem dimer was also constructed and designated pVLSNOGMI2 (SEQ ID NO:16).

Oligonucleotides (SEQ ID NO:53 and 54) containing a splice acceptor (SA) of AKV virus (in bold) was inserted into pVLSNOGMI at the unique *Sac* 2 site just before the IRES, resulting in pVLSNOGMIS (SEQ ID NO:15).

Oligo: (SEQ ID NO:53)

5' -GGCCGCT**AACTAATAGCCCATTCTCCAAGGTACGTAGC**-3'



3' -CGCCGGCGATTGATTATCGGGTAAGAGGTTCCATGCAT-5'

(SEQ ID NO:54, bottom Oligo)

### Recovery of LTR promoter sequences from mouse CD4+ T-helper cells

5 In order to facilitate the recovery of VL30 promoter sequences expressed in mouse T-helper cells, a mouse CD4+ T-helper cell cDNA library (Stratagene, San Diego, CA, Catalog # 937311) was screened by plaque hybridization. Approximately  $2 \times 10^4$  bacteriophage  $\lambda$ -ZAP clones were plated on a lawn of *E. coli* cells according to the manufacturer's instructions. Two nylon filters were sequentially layered onto the lawn of *E.*  
10 *coli* cells and bacteriophage. The filters were hybridized to a  $^{32}\text{P}$ -labelled (Prime-It RmT Random Primer Labeling Kit, Stratagene), 4.2 kb internal *Xho*I fragment of NVL-3 (containing the NVL-3 genome). 55 plaques (or approximately 0.3% of the total phage) reacted positively on both filters. 18 VL30 cDNA sequences were cloned from the plate, which was used to identify U3 promoters that are actively expressed in the RNA of mouse T-  
15 cells. Five of the 18 clones contained intact U3 sequences, representing four of one molecular species, named TH1 (SEQ ID NO: 2) and one of another species, named TH2 (SEQ ID NO: 3) also provided in Fig. 5. TH1 contained approximately 120 bp more DNA than did TH2. Because TH1 was more abundant (4 out of 5 clones), the additional sequences in the enhancer region were implicated to be a possible reason for the stronger expression in  
20 mouse T cells. Examination of the known and putative transcription factor binding sites in the VL30 LTR (Hodgson, 1996, chapter 4, Fig. 4.2 *supra*) revealed several interesting features of TH1 and TH2. First, the extra sequences of TH1 that were missing in TH2 included an extra copy of the enhancer repeat region as well as a potential retinoid (RAR/RXR) binding site. Several transcription factor binding sites in the enhancer repeat  
25 region that differed between the two elements included: a cyclic 3'-5'AMP response element (VLCRE, a potential CREB/jun binding site), a serum response element (SRE), and a potential NF1/IL6 binding site (although there were additional sites for these factors in other enhancer repeats). These factors could possibly explain why VLTH1 appeared to be expressed at higher levels, both in the source cells and into transduced cells. Together, the  
30 VL30 sequences represented 0.3% of the mRNA expressed in the T cells, and TH1 appeared to be most abundant VL30.

## Sequencing Primers:

(SK, SEQ ID NO:49) 5'-CGCTCTAGAACTAGTGGATC (20 mers, T<sub>m</sub> 60°C).(T7, SEQ ID NO:50) 5'-GTAATACGACTCACTATAGGG (21 mers, T<sub>m</sub> 60°C).5      **Seamless Rescue of T cell promoters using class IIS restriction enzymes**

Two sets of primers containing offset *Bpm*1 restriction sites were designed and synthesized. One set was for amplification of the plasmid sequences, and another was for the amplification of the inserts.

10      **Insert Primers: (Bpm1 site bold)**ITA (43 mer, T<sub>m</sub>: 67.2 °C, SEQ ID NO:45)CGATCCACT**TGGAG**CTCGGAGCCCACCCCCTCCCATCTAGAGGT15      ITB (43 mers, T<sub>m</sub>: 66.3 °C, SEQ ID NO:46)CGTCCTCCT**TGGAG**AGCACAGGGTAGAGGAGTCTCGACGGTCAG**Vector primers: (Bpm1 site bold)**VLA (43 mers, T<sub>m</sub>: 68.2 °C, SEQ ID NO:47)CGCAACCCT**TGGAG**ACCTCTAGATGGGAGGGGGTGGGCTCCGAG20      VLB (43 mers, T<sub>m</sub>: 66.3 °C, SEQ ID NO:48)GCAGGACCT**TGGAG**CTGACCGTCGAGACTCCTCTACCCTGTGCT

To amplify vector sequences more efficiently, vector templates were shortened by deleting marker genes from vectors. pVLSNOSIB (SEQ ID NO:14) was cut with *Kpn* 1 and a 4201 bp fragment containing β-gal gene was removed. The remaining vector has 3923  
25      bp.

The U3-promoter inserts (357 bp for TH1 and 240 bp for TH2) were PCR-amplified from TH1 and TH2 promoters with primers ITA and ITB. The vector cassettes (~4.2 kb for pVLSNOSIB and ~3.7 kb for pVLSNOGMIS) were PCR-amplified from the shortened vector templates using primers VLA and VLB, (*supra*). The PCR-amplification  
30      was done with high-fidelity *Pfu* DNA polymerase from Stratagene (La Jolla, CA). The amplified products were gel-purified (1% agarose gel). The inserts were then cut with *Bpm* 1 to produce complementary ends. The vector cassette products were phosphorylated with

PNK, then circularized with T4 ligase, and transformed into SCS 110 cells. Recovered plasmids were then digested with *Bpm* 1 and treated with CIP to produce complementary ends. *Bpm* 1 treated inserts and vector cassettes were ligated, and T-cell tissue-specific VL 30 vectors VLTH1 and VLTH2 were produced. The marker  $\beta$ -gal gene and GFP gene were put  
5 back into those vectors at the original unique sites *Kpn* 1 and *Sal* 1 respectively.

### Transmission and expression of single LTR vectors and T cell U3 sequences

Vector DNA constructs were transfected into GP+E86 retroviral helper cells (Markowitz et al, 1988, *supra*) using the Lipofectamine protocol (Life Technologies,  
10 Gaithersburg, MD). The culture media from these cells (supernatant), containing defective transducing particles (72 h post-transfection), was transmitted to PA317 (Miller, US Patent, cited *supra*) amphotropic helper cells, using Lipofectamine to enhance transduction efficiency (Hodgson *et al.*, 1996. Synthetic Retrotransposon Vectors and Gene Targeting pp. 3-14, in : Felgner et al., eds. *Artificial Self-Assembling Systems for Gene Delivery*. American Chemical  
15 Soc. Books, Washington, D.C.). A similar procedure was used to transmit VLTH1 and VLTH2 to the PG13 helper cell line (Miller *et al.*, 1991. *J. Virol.* 65:2220-2224). 24 h post-transfection, the recipient cells were selected with the drug G418 (500 $\mu$ g/ml, 2 weeks) to enrich for stably transduced cell populations.

All of the single LTR vectors, including VLTH1 and VLTH2 were transmitted  
20 by this method, indicating that single LTR vectors can be used for promoter switching and yet revert to dual LTR vectors after a single passage. Vectors VLSNO2, VLSNO3, and VLSNOSIB were then titrated on NIH 3T3 cells (using the PA317 vector producer cell lines). VLTH1 and VLTH2 vectors were titrated on human HT1080 cells (PG13 cell lines). Surprisingly, all of the single LTR vectors were transmitted effectively. However the titers of  
25 stably transduced TH1 and TH2 cell lines were  $5.5 \times 10^2$ - $1.1 \times 10^3$  TU/ml, compared to  $0.4$ - $3.0 \times 10^4$  TU/ml for the VLSNO2, VLSNO3 and VLSNOSIB cell lines. Thus, switching from the NVL-3 transcriptional promoter (originally isolated from NIH 3T3 fibroblast cells) to VL30 promoters derived from T helper cells, appeared to have a negative effect on RNA expression in fibroblast cells, as determined by the transmissibility of the RNA.

30 In order to study the usefulness of rescued promoters as DNA transfection vectors (as opposed to retro-vectors), VLSNOSIB, VLTH1 and VLTH2 were also transfected into a number of cell lines (using Lipofectamine), including NIH 3T3, PA317, GP+E86,

PG13, HT1080, SW480 and HeLa (available from ATCC). RNA expression in these cell lines is shown in Table 4, wherein gene expression from the LTR promoter (as determined by  $\beta$ -gal staining) is normalized to VLSNOSIB (100).

Cell line:	NIH 3T3	PA317	GP+E86	PG13	HT1080	SW480	HeLa
<b>Vector:</b>							
<b>VLSNOSIB</b>	100	100	100	100	100	100	100
<b>VLTH1</b>	39.3	18.7	0.1	21	25.5	156	156
<b>VLTH2</b>	28.6	7.1	5.5	11.5	46.8	82	156

Table 4. Transient expression of a  $\beta$ -gal marker gene by three VL30 promoters: NVL-3 (VLSNOSIB), VLTH1 and VLTH2. Cells were transfected using the Lipofectamine procedure. Total blue cells were counted from each well in 6-well plates, and the number of blue cells from VLSNOSIB was normalized to 100%.

The expression of both the VLTH1 and VLTH2 promoters was significantly reduced compared to VLSNOSIB in cell lines of fibroblastic origin, whereas in SW480 colorectal cancer cells and HeLa cells, it was comparable to or better than VLSNOSIB (the NVL-3 promoter). However, VLSNOSIB was expressed poorly in the non-fibroblastic cell lines, so a direct comparison was difficult to interpret. Unfortunately, the human T cell lines (Jurkat and MOLT4 [obtained from ATCC]) were not transfected by Lipofectamine, and they were poorly transduced by VLTH1 and VLTH2 retro-vectors. In the Jurkat and MOLT4 cells transduced with VLTH1 and VLTH2, only a small percentage (1-10%) of cells that were stably transduced by the vectors stained positively for  $\beta$ -gal expression. However, the marker gene (neo) continued to be expressed from an internal promoter, as evidenced by drug selection.

Taken together, the results demonstrated: 1) the ability of the promoter rescue technique to seamlessly capture functional transcriptional promoters from specialized cells; 2) the ability of single LTR vectors to introduce the rescued promoters into standard transducing vectors; 3) the ability of the rescued promoters to be expressed at differing levels in several different cell types, including T cells; and 4) screening and selection established the efficacy, or lack thereof, of individual promoter sequences.

Although the general method of promoter rescue was demonstrated by the foregoing experiments, the titers obtained from the sLTR VL30 vectors may not be useful where selection systems are not available.

Additional experimentation led to the development of a chimeric packaging signal, combining the essential packaging signal from Moloney murine leukemia virus ( $\Psi$ ), and the enhanced packaging signal ( $\Psi^+$ ) from a mouse VL30 element. A vector embodiment of this packaging system is VLMB (SEQ ID NO:23). One advantage of the chimeric packaging system was the elimination of retroviral *gag* gene sequences that were present in previous high-titer MLV-based vectors (viral *gag* sequences contribute to the generation of replication competent retrovirus outbreaks). The titers of VLMB-based vectors ranged from approximately  $1 \times 10^5$  to  $4 \times 10^6$  TU/ml.

#### Construction of a cloning vector for promoter rescue

Using pVLSNOGMIS as a template, and primers (SEQ ID NOS:28 and 68), a 6.4 kb plasmid fragment was PCR amplified (Using Hot Start Ampliwax PCR Gems 100, Perkin Elmer). 30 cycles of PCR were performed by following the manufacturer's instructions, with the following input conditions: lower reaction,  $80^\circ \text{C}$ , 5 min., then add upper reaction and template,  $96^\circ \text{C}$ , 1 min. Each reaction vial contained 50 ng template, 0.5  $\mu\text{M}$  each primer, 200  $\mu\text{M}$  dNTPs and 5U (2 $\mu\text{l}$ ) *Pfu* polymerase (Stratagene, LaJolla, CA). 30 repeating cycles of:  $96^\circ \text{C}$ , 45 sec;  $50^\circ \text{C}$ , 45 sec;  $75^\circ \text{C}$ , 1 min. A final incubation of  $75^\circ \text{C}$ , 10 min, then hold at  $4^\circ \text{C}$ . After amplification, the reactions were purified using Qiaquick PCR Purification Kits (Qiagen). The PCR products were digested with *Pac1*, heat inactivated ( $65^\circ \text{C}$ , 20 min) and ligated together using T4 DNA ligase (overnight at  $4^\circ \text{C}$  in a 5  $\mu\text{l}$  vol). The ligated DNA was transfected into SCS110 *E. coli* cells (Stratagene) with kanamycin (50  $\mu\text{g}/\text{ml}$ ) antibiotic added to the agar plates. The cells were *dcm*<sup>-</sup>, *dam*<sup>-</sup> (to prevent methylation of *Bpm1* sites). The resulting plasmid, pVLBPGN (SEQ ID NO:1, Figs 2 &3) has a deletion in the U3 region of the LTR. A linker containing a central *Pac1* site flanked by two outwardly-digesting *Bpm1* sites occupies the site of the deleted U3 sequences. The *Bpm1* sites enable the plasmid to be digested with *Bpm1*, resulting in two 2 bp 3'-overhanging ends that are complementary to the U3-derived RT-PCR inserts described below. The digested plasmid was purified free from the intervening linker sequences from an agarose gel after digestion with *Bpm1*, using the Qiaquick gel purification kit (Qiagen).

### Procedure for amplification of liver U3 promoter region

Purified mouse liver total tissue RNA was purchased from Ambion, Inc., (Austin, TX). Total liver RNA was treated with RQ1 Rnase-free (Promega, Madison, WI). Using Perkin Elmer Gene Amp thermostable rTth reverse transcriptase RNA PCR kit (P/N N808-0069), the following conditions for RT-PCR were used: RT-PCR A 70° (hot start); RT-PCR B, 95°C, 60 sec, then 35 cycles (95°C 10 sec, 58°C, 15 sec) then a final 58°C incubation for 7 min, then 4°C and hold. Additional conditions were: primer concentration 0.15 micromolar, template 100 ng/reaction, dNTPs 200 micromolar (final) and MgCl<sub>2</sub> 3.5 mM(final). The primers for insert amplification were SEQ ID NOS:28 and 68)

The amplified U3 sequences were purified using Qiaquick. The pVLBPGN plasmid was digested with *Bpm1*, isolated from a 1% agarose gel and purified using the Qiaquick method. The purified U3 sequences were ligated at 1:2, 1:4 and 1:6 molar ratios of VLBPGN plasmid:insert using T4 DNA ligase and a 5 microliter reaction volume overnight at 4°C (100 ng plasmid: 16 ng insert = 1:1 molar ratio). 1 microliter of each ligation reaction was transformed into *E. coli* SCS 110 competent cells (Stratagene). 26 colonies were recovered in total. Out of 23 clones grown overnight in the presence of kanamycin, 20 had sequences that appeared to be mouse VL30 sequences, representing 10 different VL30 species (Fig. 6, SEQ ID NOS: 4-13). One of these (Hep 10, SEQ ID NO: 13) was transiently transfected into Hep G2 liver hepatocellular carcinoma cells. 48 h after transfection, intense GFP fluorescence was observed, indicating strong expression of the Hep 10 U3 promoter region.

### Example 4

#### Creating a combinatorial library of mouse VL30 U3 sub-regions.

Using Fig. 7 and Hodgson, 1996, supra, Fig. 4.2 as a guide, the following three sub-regions of the VL30 U3 region were empirically established: Distal (1); medial (2); and proximal (3). Peaks of similarity were used to guide the following choice of primers: (+) primer binding site-5'-LTR boundary; ~80 bp (defines sub-region 1); ~80-210 bp (sub-region 2); ~210-430 (sub-region 3). The following primers were selected to amplify the vector VLBPGN or a similar VL30, NVL-3 LTR-containing vector:

P1 (going left from the 5'-end of the LTR to amplify the plasmid)

(SEQ ID NO:55)

GACTAACCTTGATTCCACTGGAGTTTT(CT)(CT)ATTCTTCATTCCCCACTTC  
TTCTT

P2 (going right from the 3'-end of the promoter region to amplify the plasmid)

5 (SEQ ID NO:56)

GACTAACCTTGATTCCACTGGAGAATCTGGACCAATTCTATATAAGCCTG  
TGAAAAATTT

The six primers selected to amplify the inserts are as follows:

10 Fragment 1, primer 1 (going right from the LTR terminus into U3) (SEQ ID NO:57)

GACTAACCTTGATTCCACTGGAGAAGAAGAAGTGGGGAATGAAGAA

Fragment 1, primer 2 (going left from the end of fragment 1) (SEQ ID NO:58)

GACTAACCTTGATTCCACTGGAGATCTCTAGATGGGAGGGG(GT)(CT)GGG  
CTC

15 Fragment 2, primer 1 (going right from the left end of fragment 2) (SEQ ID NO:59)

GACTAACCTTGATTCCACTGGAGCTCGGAGCCCACCCCCTCCCATCT

Fragment 2, primer 2 (going left from the right end of fragment 2) (SEQ ID NO:60)

GACTAACCTTGATTCCACTGGAGGGAGGCCCTTATCTCAAAAATGTT

Fragment 3, primer 1 (going right from the left end of fragment 3) (SEQ ID NO:61)

20 GACTAACCTTGATTCCACTGGAGTCTAAGAACATTTTTGAGATAAGGGCC  
T

Fragment 3, primer 2 (going left from the right end of fragment 3) (SEQ ID NO:62)

GACTAACCTTGATTCCACTGGAGTCACAGGCTTATATAG(TG)AAA

25 100 ng of genomic DNA from *Mus musculus* is used as a template (the mouse genome bears 100-200 copies of VL30 elements). Standard PCR procedures for *Pfu* polymerase are used. Fragments are amplified 35 rounds of PCR to obtain single-copy genomic DNA amplification. Samples of Qiagen column purified DNA are examined on analytical agarose gels to determine the approximate size. The remainder of each reaction is digested with the  
30 appropriate enzyme and run on an acrylamide or agarose gel. The digested fragments are purified by standard gel purification procedures and are ligated to the plasmid fragment at an equimolar ratio of the four PCR fragments (three inserts and one plasmid). The ligation mix

is transformed into *E. coli* SCS1 and is grown on kanamycin. The number of colonies is used to establish the size of the combinatorial library, and the pooled colonies are grown in *E. coli* and the DNA is harvested *en masse*. A dozen or more colonies are characterized by DNA sequencing to determine the approximate fidelity of the reaction. A library of 1,000 or more,  
5 but preferably 100,000 or more members is used for combinatorial screening procedures.

**Screening the combinatorial libraries for expression in specific cell types using a replication defective helper virus**

The U3 library DNA is transfected into the desired target cells in which  
10 expression is desired. Along with the library, approximately 25% of the total DNA should include retroviral helper sequences. The latter sequences can be a helper plasmid (such as pPAM3, Miller *et al.*, US Patent 4,861,719). The virus is amphotropic, permitting it to infect most human cells. The RNA from individual clones that are transcribed in the target cells will be packaged into retroviral virions made by the helper virus, and the virions can be harvested  
15 as the cell free filtrate (0.45 mm) from the vector producer cells. This virus (containing the expressed sequences) can be transmitted to fresh target cells that do not contain helper virus. 48 hours after passage, the DNA form of the transcriptionally active clones will be integrated in the recipient cells, and these transcriptionally active loci will produce more RNA, and protein. After G418 drug selection to increase the proportion of cells expressing the vector  
20 sequences, helper virus DNA is again transfected into the recipient cells, transforming them into vector producer cells. The virus from these cells should contain increased amounts of the RNA from clones that are transcriptionally active in those cells. Passage of the virus is continued for two or three rounds to permit recombination and mutation to take place, enhancing the effect of *in vitro* evolution of promoters. The actual degree of enhancement  
25 attainable at each step is illustrated in Table 2 (*supra*). After several passages, the actual level of RNA expressed by several clones is determined by RNA blotting, or by the amount of a reporter gene expressed as protein (determined visually or by the appropriate assay). Because human cells do not naturally contain VL30 DNA or RNA, the sequences that remain in the human cells are those with the most transcriptionally active promoters. These  
30 sequences can be amplified and re-cloned using the methods of the instant invention, or they can be rescued by virus packaging, reverse transcribed by the endogenous reverse



transcriptase reaction, and grown as plasmids (due to their plasmid origin of replication and the selectable kanamycin marker gene).

In addition to using a replication defective helper virus, such as the clone pPAM3, it is also possible to use a replication competent retrovirus, such as Moloney murine leukemia virus to passage the library. For use in human cells, however, the virus should have a tropism that is compatible with human cells (gibbon ape leukemia virus and amphotropic [4070A] murine retroviruses are acceptable).

In addition to being useful for generating active transcriptional promoters *de novo*, a small variation on the above procedures may enable the isolation of hormone responsive promoters. In it, the cells are treated with the hormone (which could be a steroid, a peptide hormone known to affect the cells, a drug, a drug agonist or antagonist, etc.) during passage. After isolation of surviving VL30 vector-containing cells, individual clones of drug resistant cells are tested for reporter gene expression with and without drug treatment to determine relative protein expression. Likewise, RNA expression can be determined by blot analysis or a similar method. A useful list of known VL30 responses to pharmacological agents is listed in Fig. 4.2 of Hodgson, 1996, *supra*, and can be used as a guide to help assess the potential agents known to have an effect on VL30 transcription.

Once the transcriptional promoters with the known specificity have been obtained, they can be used to obtain expression of genes from a variety of types of vectors. For example, in addition to retrovirus particles, the promoters can be incorporated into all other major groups of vectors: adenoviruses, herpes simplex virus vectors, DNA transfection vectors, etc. It will be apparent to persons of ordinary skill in the art that similar combinatorial libraries can also be used to screen for other characteristics than transcription activity in a particular cell. For example, combinatorial libraries of complementarity determining regions (CDRs) of antibodies or T cell receptors can be so screened using antibody screening methods, such as the phage display screening method (Pharmacia, Milwaukee, WI). Thus, the methods of this invention, particularly the combinatorial simplicity of this invention is a significant improvement over many *in vivo* recombination methods including those of (Stemmer, US Patent 5,605,793; 1997) that have described for the production of CDR combinatorial libraries.

### **Example 5 Gene Assembly Line**

5                   From the above examples of 3 and 6 fragment gene self-assemblies, it is evident that assembly of genes by means of gene amplification, the use of offset restriction enzymes and incorporating unique, non-palindromic ends is a highly efficient process compared to conventional cloning methods. However, in addition to the considerations already discussed, it will be apparent to a person of ordinary skill in the art that the various  
10                   procedures, protocols, methods and material of the instant invention become more difficult to use as the number of fragments increases. For example, if the efficiency of combining each fragment in an assemblage is 99%, then the overall efficiency of combining ten fragments will be 90%, the efficiency of combining 100 fragments will be 37%, etc. Therefore, a small drop in efficiency of any step or fragment, or a large increase in the complexity of the project,  
15                   will be sufficient to reduce the overall efficiency. Fastidious procedures permit one to achieve success with more complex projects.

                  Foremost in its potential for inducing failure is human error in primer design where large numbers of fragments are used. Fortunately, the instant invention is suited to automation of most of the steps. This allows human input to be focused on design, analysis,  
20                   and quality control. For the purposes of generating large vectors or chromosomes, it is desirable to provide an automated environment. One method to achieve this goal is a gene assembly line.

                  In a gene assembly line, multiple tasks are controlled by a machine or machines working together to increase speed and efficiency and to reduce human error. For  
25                   example, computer aided design (CAD) and computer aided manufacturing (CAM) are incorporated and combined with the methods of this invention. The computers accept inputs in the form of template and primer sequences, together with preferences of regions to be copied and joined. The preferences include at least the sequences of the primer regions and information about the known restriction sites and maps of the sequences to be assembled, but  
30                   ideally include the entire sequence. The preferences also include the number of sequences to be joined, the desired  $T_m$  for the primers, the list of potential restriction enzymes capable of offset digestion that are potential candidates for use in the assembly process, the desired end structures for each fragment terminus, a tag sequence (if any), whether circular or linear ends

are desired, and additional design considerations. The computer algorithm then searches the sequences to determine the candidate enzymes and specific primers that match the criteria of the input. Candidates for selection of unique non-palindromic overlaps are selected. The computer then posts selections or preferences for the type and order of end structures, the primer binding sites, their potential for primer-dimer and intra-molecular interaction artifacts, and the potential conflicts with repeat sequences within the templates that could lead to incorrect polymerization. Based upon the selections made by the operator, the computer then determine the  $T_m$  for each primer, and makes adjustments (with suitable inputs from the investigator) to achieve a suitable  $T_m$  for the appropriate DNA synthesis or gene amplification reaction. Ideally, the primers should have similar  $T_m$ s so that all amplification reactions can be performed at once with one set of amplification instructions. In reality, it may be difficult to do this with complex projects. The output of this portion of the program, which can be in a generic format, such as a Microsoft Excel spreadsheet is then downloaded to a computerized oligonucleotide synthesizer, such as the Applied Biosystems 3928 nucleic acid synthesizer. One advantage of using a computerized synthesizer is its robotic capability to de-protect and purify the oligonucleotides automatically. In addition this synthesizer can accept computerized input.

The quantity of individual oligos recovered is then determined spectrophotometrically. It is desirable to purify the oligonucleotides by high performance liquid chromatography or by polyacrylamide gel. In a preferred embodiment, the oligonucleotides and templates are then assembled robotically using an automated nucleic acid handling system such as the Qiagen BioRobot 9600. The BioRobot is capable of accepting input from a computer and can combine the gene amplification reactions based upon the assignments of templates, primer and reagents provided in the input. The assembled reactions are then amplified for example by PCR. In a preferred embodiment, the PCR heat block is incorporated into the robotic workspace and genes are assembled robotically but with minimal human intervention to change buffers, rearrange the platform, change programs, and the like. The resulting amplified products are also purified by the BioRobot or a similar robotic device. In a preferred embodiment, the robotic device uses Qiaquick cleanup procedures, or a similar method and then assembles restriction endonuclease reactions to digest the purified gene amplification products. The gene amplification products are loaded onto a gel and electrophoresed. Human intervention may be necessary to analyze the

products and excise the correct fragments from the gel. At this point, the results are assessed and missing or incorrect sized fragments are resynthesized. The robotic device is preferably used to purify the gel fragments using Quiagen or similar cleanup procedures. After spectrophotometric quantitation of the purified fragments, the robotic device is preferably  
5 used to assemble the ligation. Ideally the fragments are combined in an equimolar ratio of 1:1. However it is not necessary to use equimolar ratios in order to achieve gene self-assembly. For automated gene assembly, it may be desirable not to use equimolar ratios of input fragments, particularly if it simplified the task of quantitation. After ligation, the assemblies can be purified and ethanol precipitated or they can be added to the appropriate  
10 host cells. Automation aids in maintaining the sterility of the reaction.

Several additional considerations can assist in the construction of long genes using gene assembly. First the number of fragments and the length of constructs are limiting factors. In addition to maintaining high standards of purify of both the oligonucleotide primers and gene amplification products, it is important to keep the error rate low during  
15 copying. Thus, one can optimally start with 100 ng of template use only five rounds of gene amplification and finish with nearly 2 micrograms of product. This is more desirable for reducing errors than using a large number of amplification steps. It is also desirable to use a special copying enzyme such as *Pfu* DNA polymerase that has a low intrinsic error rate. Further it is desirable to use *in vivo* selection (in eukaryotic cells or tissues) rather than *E. coli*  
20 cloning to reduce the incorporation of errors into the vectors. For example, a viral vector such as an adenoviral vector or the retro-vectors of the preceding examples are auto-selecting. A single correctly-assembled adenovirus vector molecule, for example, leads to a lytic infection (the viral products of which are cloned by limiting dilution on the appropriate eukaryotic cells), even though it may be combined in a ligation mix with a large excess of  
25 incorrectly assembled molecules that are non-functional. Thus, it is not necessary to have a high efficiency, although high efficiency has been demonstrated in this system, in order to achieve success in making, for example gene therapy vectors.

For long fragments (3-30 kb), it is desirable to use enzymes and procedures that are designed or facilitate replication of long fragments, one such example is the  
30 eLONGase system (Life Technologies). This system can copy up to 30 kb on a fragment with proofreading. Considerations for long PCR are reviewed in Beck, 1998. (The Scientist 6 January, 1998, pp. 16-18).

Internal restriction sites are a potential problem, particularly with large constructs and can be overcome in a number of ways. Use of alternate enzymes, methylation of internal restriction sites (such as by using methylated DNA precursors during synthesis to leave the sites in primers unaffected, incorporation of the internal sites into the construct (if they are non-palindromic), or mutagenesis of internal sites, are exemplary ways to deal with some of these issues.

For very large constructs, it is desirable to use enzymes such as *SapI* (recognizing 7 nucleotides and leaving a 3 bp overhang). This enzyme digests every 16,384 bp on average. There are 64 nucleotide triplet combinations, meaning that up to 32 fragments can be ligated in a circle using *SapI*. *FokI* and *HgaI* are other examples of class IIS enzymes that are useful for making large constructs. *HgaI* has 5 bp overhangs, permitting more than 500 *HgaI* fragments to be ligated. *FokI* includes a Kozak ATG start codon. In a preferred embodiment, a *FokI* site is inserted at the PuXXATG start site of a cDNA encoding region. The cDNA is inserted in frame, providing a site for inserting and switching coding sequences within a vector.

It will be readily understood by those skilled in the art that the foregoing description has been for purposes of illustration only and that a variety of embodiments can be envisioned without departing from the scope of the invention. Therefore, it is intended that the invention not be limited except by the claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: NATURE TECHNOLOGY CORPORATION, ET AL.

(ii) TITLE OF INVENTION: SELF-ASSEMBLING GENES, VECTORS AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 68

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: MUETING, RAASCH & GEBHARDT, P.A.  
(B) STREET: 119 NORTH FOURTH STREET, SUITE 203  
(C) CITY: MINNEAPOLIS  
(D) STATE: MINNESOTA  
(E) COUNTRY: USA  
(F) ZIP: 55401

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: Not Assigned  
(B) FILING DATE: 28-FEB-1998  
(C) CLASSIFICATION:

## (vii) PRIORITY APPLICATION DATA:

(A) APPLICATION NUMBER: 60/070,910  
(B) FILING DATE: 28-FEB-1997  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: MCCORMACK, MYRA M.  
(B) REGISTRATION NUMBER: 36,602  
(C) REFERENCE/DOCKET NUMBER: 228.00010201

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 612-305-1225  
(B) TELEFAX: 612-305-1228

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6225 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGAAGAATAA	AAAATTACTG	GCCTCTTGTG	AGAACATGAA	CTTTCACCTC	GGAGCCCACC	60
CCCTCCCATC	TGGAAAACTC	CAGTTATAAC	TGGAGTTTTT	CCTTTAAAAG	CTTGTGAAAA	120
ATTTGAGTCG	TCGTCGAGAC	TCCTCTACCC	TGTGCAAAGG	TGTATGAGTT	TCGACCCCAG	180

	AGCTCTGTGT	GCTTTCTGTT	GCTGCTTTAT	TTCGACCCCA	GAGCTCTGGT	CTGTGTGCTT	240
5	TCATGTCGCT	GCTTTATTAA	ATCTTACCTT	CTACATTTTA	TGTATGGTCT	CAGTGTCTTC	300
	TTGGGTACGC	GGCTGTCCCG	GGACTTGAGT	GTCTGAGTGA	GGGTCTTCCC	TCGAGGGTCT	360
	TTCATTTGGT	ACATGGGCCG	GGAATTGAG	AATCTTTCAT	TTGGTGCATT	GGCCGGGAAT	420
10	TCGAAAATCT	TTCATTTGGT	GCATTGGCCG	GGAAACAGCG	CGACCACCCA	GAGGTCCTAG	480
	ACCCACTTAG	AGGTAAGATT	CTTTGTTCTG	TTTTGGTCTG	ATGTCTGTGT	TCTGATGTCT	540
15	GTGTTCTGTT	TCTAAGTCTG	GTGCGATCGC	AGTTTCAGTT	TTGCGGACGC	TCAGTGAGAC	600
	CGCGCTCCGA	GAGGGAGTGC	GGGGTGGATA	AGGATAGACG	TGTCCAGGTG	TCCACCGTCC	660
	GTTCGCCCTG	GGAGACGTCC	CAGGAGGAAC	AGGGGAGGAT	CAGGGACGCC	TGGTGGACCC	720
20	CTTTGAAGGC	CAAGAGACCA	TTTGGGGTTG	CGAGATCGTG	GGTTCGAGTC	CCACCTCGTG	780
	CCCAGTTGCG	AGATCGTGGG	TTCGAGTCCC	ACCTCGTGTT	TTGTTGCGAG	ATCGTGGGTT	840
25	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	CGTGGGTTCG	AGTCCCACCT	CGTGTTTTGT	900
	TGCGAGATCG	TGGGTTCGAG	TCCCACCTCG	CGTCTGGTCA	CGGGATCGTG	GGTTCGAGTC	960
	CCACCTCGTG	CAGAGGGTCT	CAATTGGCCG	GCCTTAGAGA	GGCCATCTGA	TTCTTCTGGT	1020
30	TTCTCTTTTT	GTCTTAGTCT	CGTGTCCGCT	CTTGTTGTGA	CTACTGTTTT	TCTAAAAATG	1080
	GGACAATCTG	TGTCCACTCC	CCTTTCTCTG	ACTCTGGTTC	TGTCGCTTGG	TAATTTTGT	1140
35	TGTTTACGTT	TGTTTTTGTG	AGTCGTCTAT	GTTGTCTGTT	ACTATCTTGT	TTTTGTTTGT	1200
	GGTTTACGGT	TTCTGTGTGT	GTCTTGTGTG	TCTCTTTGTG	TTCAGACTTG	GA CTGATGAC	1260
	TGACGACTGT	TTTTAAGTTA	TGCCTTCTAA	AATAAGCCTA	AAAATCCTGT	CAGATCCCTA	1320
40	TGCTGACCAC	TTCCTTTCAG	ATCAACAGCT	GCCCTTACTC	GAGCTCAAGC	TTCGAATTCT	1380
	GCAGTCGACG	GTACCGCGGC	CGCTAACTAA	TAGCCCATTC	TCCAAGGTAC	G TAGCGGGGA	1440
45	TCAATTCCGC	CCCCCCCCTA	ACGTTACTGG	CCGAAGCCGC	TTGGAATAAG	GCCGGTGTGC	1500
	GTTTGTCTAT	ATGTTATTTT	CCACCATATT	GCCGTCTTTT	GGCAATGTGA	GGGCCCCGAA	1560
	ACCTGGCCCT	GTCTTCTTGA	CGAGCATTCC	TAGGGGTCTT	TCCCCTCTCG	CCAAAGGAAT	1620
50	GCAAGGTCTG	TTGAATGTCG	TGAAGGAAGC	AGTTCCTCTG	GAAGCTTCTT	GAAGACAAAC	1680
	AACGTCTGTA	GCGACCCTTT	GCAGGCAGCG	GAACCCCCCA	CCTGGCGACA	GGTGCCTCTG	1740
55	CGGCCAAAAG	CCACGTGTAT	AAGATACACC	TGCAAAGGCG	GCACAACCCC	AGTGCCACGT	1800
	TGTGAGTTGG	ATAGTTGTGG	AAAGAGTCAA	ATGGCTCTCC	TCAAGCGTAT	TCAACAAGGG	1860
	GCTGAAGGAT	GCCCAGAAGG	TACCCCATTT	TATGGGATCT	GATCTGGGGC	CTCGGTGCAC	1920
60	ATGCTTTACA	TGTGTTTAGT	CGAGGTTAAA	AAAACGTCTA	GGCCCCCCGA	ACCACGGGGA	1980
	CGTGGTTTTT	CTTTGAAAAA	CACGATACGG	GATCCACCGG	TCGCCACCAT	GGGTAAAGGA	2040
65	GAAGAACTTT	TCACAGGAGT	TGTCCCAATT	CTTGTTGAAT	TAGATGGTGA	TGTTAATGGG	2100
	CACAAATTTT	CTGTCAGTGG	AGAGGGTGAA	GGTGATGCAA	CATACGGAAA	ACTTACCCTT	2160

	AAATTTATTT	GCACTACTGG	AAACTACCT	GTTCCATGGC	CAACACTTGT	CACTACTTTC	2220
	ACTTATGGTG	TTCAATGCTT	TTCAAGATAC	CCAGATCATA	TGAAACGGCA	TGACTTTTTTC	2280
5	AAGAGTGCCA	TGCCCCAAGG	TTATGTACAG	GAAAGAACTA	TATTTTTTCAA	AGATGACGGG	2340
	AACTACAAGA	CACGTGCTGA	AGTCAAGTTT	GAAGGTGATA	CCCTTGTTAA	TAGAATCGAG	2400
10	TTAAAAGGTA	TTGATTTTAA	AGAAGATGGA	AACATTCTTG	GACACAAATT	GGAATACAAC	2460
	TATAACTCAC	ACAATGTATA	CATCATGGCA	GACAAACAAA	AGAATGGAAC	CAAAGTTAAC	2520
	TTCAAAATTA	GACACAACAT	TGAAGATGGA	AGCGTTCAAC	TAGCAGACCA	TTATCAACAA	2580
15	AATACTCCAA	TTGGCGATGG	CCCTGTCCTT	TTACCAGACA	ACCATTACCT	GTCCACACAA	2640
	TCTGCCCTTT	CGAAAGATCC	CAACGAAAAG	AGAGACCACA	TGGTCCTTCT	TGAGTTTGTA	2700
20	ACAGCTGCTG	GGATTACACA	TGGCATGGAT	GAACATACAA	AGTCCGGATC	TAGATAACTG	2760
	TATCGATGGA	TCCGAAGGCG	GGGACAGCAG	TGCAGTGGTG	GACAGAAAGC	AAGTGATCTA	2820
	GGCCAGCAGC	CTCCCTAAAG	GGACTTCAGC	CCACAAAGCC	AAACTTGTGG	CTTTAATACA	2880
25	AGCTCTGTAA	ATGGTAAAAA	AAAAAAAGTC	TACACGGACA	GCAGGTATGC	TCTTGCCACT	2940
	GTACAGAGCA	ATATACAGAC	AAAGAGAACT	GTTGACATCT	GCAGAGAAAG	ACCTAAGATG	3000
30	CTGTGGCTAA	AAGAAATCAG	ATGGCAAATC	TAACCGCCCA	GGCATCCTAA	AGAGCAATGA	3060
	TCCTGACAGT	CTGAAGACTA	TCAAGTTATA	GACAAATTAA	GACTGGTAAA	AAAAACCCCTG	3120
	TATAAAATAG	TAAAAACTGA	AAAAAGAAAA	CTAGTCCTCT	CATGAGAAGA	CAGACCTGAC	3180
35	ATCTACTGAA	AAATAGACTT	TACTGGAAAA	AATATGTGTA	TGAATACCTT	CTAGTTTTTTG	3240
	TGAACGTTCT	CAAGATGGAT	AAAAGCTTTT	CCTTGTAATA	CGAGACTGAT	CAGATAGTCA	3300
40	TCAAGAAGAT	TGTTAAAGAA	AATTTTCCAA	GGTTCGGAGT	GCCAAAAGCA	ATAGTGTGAG	3360
	ATAATGGTCC	TGCCTTTGTT	GCCCAGGTAA	GTCAGGTGTG	GGCCAAGTAT	TTAGAGGTCA	3420
	AATGAAAATT	CCATTGTGTG	TACAGACCTC	AGAGCTCAGG	AAAGATAAAA	AAGAATAAAT	3480
45	AAACTCTTAA	ACAGACCTTG	ACAAAATTAA	TCCTAGAGAC	TGGCACAGAC	TTACTTGCTA	3540
	CTCCTTCCCC	TTGCCCTATT	TAGAACTGAG	AATACTCCCT	CTTGATTTCG	TTTTACTCTT	3600
50	TTTAAGATCC	TTTATGGGGC	TCCTATGCCA	TCACTGTCTT	AAATGATGTG	TTTAAACCTA	3660
	TGTTGTTATA	ATAATGATCT	ATATGTTAAG	TTAAAAGGCT	TGCAGGTGGT	GCAGAAAGAA	3720
	GTCTGGTCAC	AACTGGCTAC	AGTGAACAAG	CTGGGTACCC	CAAGGACATC	TTACCAGTTC	3780
55	CAGCCAGAGA	TCTGATCTAC	GATCCCCGGG	TCGACCCGGG	TCGACCCTGT	GGAATGTGTG	3840
	TCAGTTAGGG	TGTGGAAAGT	CCCCAGGCTC	CCCAGCAGGC	AGAAGTATGC	AAAGCATGCA	3900
60	TCTCAATTAG	TCAGCAACCA	GGTGTGGAAA	GTCCCCAGGC	TCCCCAGCAG	GCAGAAGTAT	3960
	GCAAAGCATG	CATCTCAATT	AGTCAGCAAC	CATAGTCCCG	CCCCTAACTC	CGCCCATCCC	4020
	GCCCCTAACT	CCGCCAGTT	CCGCCCATTC	TCCGCCCAT	GGCTGACTAA	TTTTTTTTTAT	4080
65	TTATGCAGAG	GCCGAGGCCG	CCTCGGCCTC	TGAGCTATTC	CAGAAGTAGT	GAGGAGGCTT	4140
	TTTTGGAGGC	CTAGGCTTTT	GCAAAAAGCT	TCACGCTGCC	GCAAGCACTC	AGGGCGCAAG	4200



	GGCTGCTAAA	GGAAGCGGAA	CACGTAGAAA	GCCAGTCCGC	AGAAACGGTG	CTGACCCCGG	4260
5	ATGAATGTCA	GCTACTGGGC	TATCTGGACA	AGGGAAAACG	CAAGCGCAAA	GAGAAAGCAG	4320
	GTAGCTTGCA	GTGGGCTTAC	ATGGCGATAG	CTAGACTGGG	CGGTTTTATG	GACAGCAAGC	4380
	GAACCGGAAT	TGCCAGCTGG	GGCGCCCTCT	GGTAAGGTTG	GGAAGCCCTG	CAAAGTAAAC	4440
10	TGGATGGCTT	TCTTGCCGCC	AAGGATCTGA	TGGCGCAGGG	GATCAAGATC	TGATCAAGAG	4500
	ACAGGATGAG	GATCGTTTCG	CATGATTGAA	CAAGATGGAT	TGCACGCAGG	TTCTCCGGCC	4560
15	GCTTGGGTGG	AGAGGCTATT	CGGCTATGAC	TGGGCACAAC	AGACAATCGG	CTGCTCTGAT	4620
	GCCGCCGTGT	TCCGGCTGTC	AGCGCAGGGG	CGCCCGGTTT	TTTTTGTCAA	GACCGACCTG	4680
	TCCGGTGCCC	TGAATGAACT	GCAGGACGAG	GCAGCGCGGC	TATCGTGGCT	GGCCACGACG	4740
20	GGCGTTCCTT	GCGCAGCTGT	GCTCGACGTT	GTCACTGAAG	CGGGAAGGGA	CTGGCTGCTA	4800
	TTGGGCGAAG	TGCCGGGGCA	GGATCTCCTG	TCATCTCACC	TTGCTCCTGC	CGAGAAAGTA	4860
25	TCCATCATGG	CTGATGCAAT	GCGGCGGCTG	CATACGCTTG	ATCCGGCTAC	CTGCCCATTG	4920
	GACCACCAAG	CGAAACATCG	CATCGAGCGA	GCACGTACTC	GGATGGAAGC	CGGTCTTGTC	4980
	GATCAGGATG	ATCTGGACGA	AGAGCATCAG	GGGCTCGCGC	CAGCCGAACT	GTTGCCCAGG	5040
30	CTCAAGGCGC	GCATGCCCCA	CGGCGAGGAT	CTCGTCGTGA	CCCATGGCGA	TGCCTGCTTG	5100
	CCGAATATCA	TGGTGGAAAA	TGGCCGCTTT	TCTGGATTCA	TCGACTGTGG	CCGGCTGGGT	5160
35	GTGGCGGACC	GCTATCAGGA	CATAGCGTTG	GCTACCCGTG	ATATTGCTGA	AGAGCTTGGC	5220
	GGCGAATGGG	CTGACCGCTT	CCTCGTGCTT	TACGGTATCG	CCGCTCCCGA	TTCGCAGCGC	5280
	ATCGCCTTCT	ATCGCCTTCT	TGACGAGTTC	TTCTGAGCGG	GACTCTGGGG	TTCGAAATGA	5340
40	CCGACCAAGC	GACGCCCAAC	CTGCCATCAC	GAGATTTCGA	TTCCACCGCC	GCCTTCTATG	5400
	AAAGGTTGGG	CTTCGGAATC	GTTTTCCGGG	ACGGAATTCG	TAATCTGCTG	CTTGCAAACA	5460
45	AAAAAACCAC	CGCTACCAGC	GGTGGTTTGT	TTGCCGGATC	AAGAGCTACC	AACTCTTTTT	5520
	CCGAAGGTAA	CTGGCTTCAG	CAGAGCGCAG	ATACCAAATA	CTGTCCTTCT	AGTGTAGCCG	5580
	TAGTTAGGCC	ACCACTTCAA	GAAGTCTGTA	GCACCGCCTA	CATACCTCGC	TCTGCTAATC	5640
50	CTGTTACCAG	TGGCTGCTGC	CAGTGGCGAT	AAGTCGTGTC	TTACCGGGTT	GGACTCAAGA	5700
	CGATAGTTAC	CGGATAAGGC	GCAGCGGTCG	GGCTGAACGG	GGGGTTCGTG	CACACAGCCC	5760
55	AGCTTGAGC	GAACGACCTA	CACCGAACTG	AGATACCTAC	AGCGTGAGCA	TTGAGAAAGC	5820
	GCCACGCTTC	CCGAAGGGAG	AAAGGCGGAC	AGGTATCCGG	TAAGCGGCAG	GGTCGGAACA	5880
	GGAGAGCGCA	CGAGGGAGCT	TCCAGGGGGA	AACGCCTGGT	ATCTTTATAG	TCCTGTCGGG	5940
60	TTTCGCCACC	TCTGACTTGA	GCGTCGATTT	TTGTGATGCT	CGTCAGGGGG	GCGGAGCCTA	6000
	TGGAAAAACG	CCAGCAACGC	CGAGATGCGC	CGCCTCGAGT	ACACCTGCGT	CATGCTGAGA	6060
	CCCTCAAGCC	TACTATAAAG	GGTCCCTGCC	TAGTTCTGTT	TACTAATCTG	CCTTATTCTG	6120
65	TTTTTGTTCC	CATGTTAAAG	ATAGAGTAAA	TGCAGTATTC	TCCACATAGA	GATATAGACT	6180

TCTGAAATTC TAAGATTAGA ATTATTTACA AGAAGAAGTG GGGAA

6225-

(2) INFORMATION FOR SEQ ID NO:2:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 487 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCTCCCATCT AGAGGTTGTT CTCGGAACAC TCCTAAACTT TTCACCCCAA AACTCCTCAC 60  
20 CCTAAAGTTC GAAAAAACTG TTCCAAGAAC ATTTTGTAGA TAAAGGCCTC CTAGAACAAC 120  
CTCAAATGA CATTGCCAAA TGATAAGACA TGACTCCTTA GTTACGTAGG TTCCTTGATA 180  
GGACATGACT CCTTAGTTAC GTAGGTTTCCT TGATAGGACA TGACTCCTTA GTTACGTAGA 240  
25 TTCCTTTGGT AGAACTCCCT AGTGATGTAA ACTTGTACTT TCCCTGCCCA GTTCTCCCCC 300  
TTTGAGTTTT ACTATATAAG CCTGTAAAAA ATTTTGTCTG ACCGTCGAGA CTCCTCTACC 360  
30 CTGTGCTAAG GTGTATGAGT TCGACCCCA GAGCTCTGTG TGCTTCCATG TTGCTGCTTT 420  
ATTTTCGACCC CAGAGCTCTG GTCTGTGTGC TTTCATGTCG CTGCTTTATT AAATCTTGCC 480  
TTCTACA 487

35 (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 366 base pairs  
40 (B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTCCCATCT AGAAAACATT TTTGAGATAA AGGCTTCCTG GAACAACCTC AAAATGAACC 60  
AGGTACTCCT TAGTTACGTA GGTTTCCTTGA TAGGACATGA CTCCTTAGTT ACATAGATTC 120  
55 CTTTGGCAGA ACTCCCTAGT GATGTAAACT TGTACTTTCC CTGCCCAGTT CTCCCCCTTT 180  
GAGTTTTACT ATATAAGCCT GTGAAAAATT TTGGCTGACC GTCGAGACTC CTCTACCCTG 240  
TGCTAAGGTG TATGAGTTTC GACCCAGAG CTCTGTGTGC TTCCATGTTG CTGCTTTATT 300  
60 TCGACCCAG AGCTCTGGTC TGTGTGCTTT CATGTTGCTG CCTTATTAAA TCTTGCCTTC 360  
TACATT 366

65 (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 304 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTCCCATCT	AGAGATTGTT	CCCAGAACAC	TCCTGAACTC	TTCACCCCAG	AATGCATGCC	60
15	TGAACTCCTC	ACCCTAGAGT	TCGAACCCTC	CCAATAAAG	ACTGTTCCAA	120
	GAGATAAGGG	CCTCCTGGAA	CAACCTCAGA	ATGAACCGGG	TACATTGCCA	180
	CATGACCCCT	TAGTTACGTA	AAATCCCTTG	GCAGAACCCC	TTGTCCCTTG	240
20	TTAGTTATGT	AAACTTGTAC	TTTCCCTACC	CCGCTCTCCC	CCCTTGAGTT	300
	AAGC					304

25 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 304 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

40	CCTCCCATCT	AGAGAGTGTT	CCCAGAACAC	TCCTGAACTC	TTCACCCCAG	AATGCATTCC	60
	TGAACTCCTC	ACCCTAGAGT	TCGAACCCTC	CCAATAAAG	ACTGTTCCAA	GAACATTTTT	120
	GAGATAAGGG	CCTCCTGGAA	CAACCTCAGA	ATGAACCGGA	TACATTGCCA	AATAATAGGA	180
45	CATGACCCCT	TAGTTACGTA	GAATCCCTTG	GCAGAACCCC	TTGTCCCTTG	GCAGAACCCC	240
	TTAGTTATGT	AAACTTGTAC	TTTCCCTACC	CCGCTCTCCC	CCCTTGAGTT	TTTCTATAT	300
50	AAGC						304

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 304 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: DNA (genomic)

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

65

CCTCCCATCT	AGAGAGTGTT	CCCAGAACAC	TCCTGAACTC	TTCACCCCAG	AATGCATTCC	60
------------	------------	------------	------------	------------	------------	----

TGAACTCCTC ATCCTAGAGT TCGAACCCTC CCAACTAAAG ACTGTTCCAA GAACATTTTT 120  
 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCTGG TACATTGCCA AATAATAGGA 180  
 5 CATGACCCTT TAGTTACGTA GAATCCCTTG GCAGAACCCC TTGTCCCTTG GCAGAACCCC 240  
 TTAGTTATGC AAACTTGTAC TTTCTCTGCC CCGCTCTCCC CCCTTGAGTT TTTCCTATAT 300  
 10 AAGC 304

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 304 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTCCCATCT AGAGAGTGTT CCCAGAACAC TCCTGAACTC TTCACCTCAA AATGCATTCC 60  
 TGAACTCCTC ACCCTAGAGT TCGAACCCTC CCAACTAAAG ACTGTTCCAA GAACATTTTT 120  
 30 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCAGG TACATTGCCA AATAATAGGA 180  
 CATGACCCTT TAGTTACGTA GAATCCCTTG GCAGAACCCC TTGTCCCTTG GCAGAACCCC 240  
 35 TTAGTTATGC AAACTTGTAC TTTCTCTGCC CCGCTCTCCC CCCTTGAGTT TTTCCTATAT 300  
 AAGC 304

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 305 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTCCCATCT AGAGATTGTT CCCAGAACAC TCCTGAACTC TTCACCCCAG AATGCATTCC 60  
 55 TGAACTCCTC ACCCTAGAGT TCGAACCCTC CCAACTAAAG ACTGTTCCAA GAACATTTTT 120  
 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCGGA TACATTGCCA AATAATAGGA 180  
 60 CATGACCCCT TAGTTACGTA GAATTCCCTT GGCAGAACCC CTGTCCCTT GGCAGAACCC 240  
 CTTAGTTATG CAAACTTGTA CTTTCCCTGC CCGCTCTCC CCCCTTGAGG TTTCCTATA 300  
 TAAGC 305

(2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 305 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCTCCCATCT AGAGAGTGTT CCCAGAACAC TCCTGAACTC TTCACCCAG AATGCATTCC 60  
TGAACCCCTC ACCCTAGAGT TCGAACCCCTC CCAACTAAAG ACTGTTCCAA GAACATTTTT 120  
GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCAGG TACATTGCCA AATAATAGGA 180  
CATGACCCCT TAGTTACGTA GAATTCCCTT GGCAGAACCC CTTGTCCCTT GGCAGAACCC 240  
CTTAGTTATG CGAACTTGTA CTTTCCCTGC CCCGCTCTCC CCCCTTGAGT TTTTCCTATA 300  
TAAGC 305

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCTCCCATC TAGAGAGTGT TCCCAGAACA CTCCTGAACT CTTTCATCCCA GAATGCATTC 60  
CTGAACTCCT CACCCTATAG TTCGAACCCCT CCCAACTAAA GACTGTTCCA AGAACATTTT 120  
TGAGATAAGG GCCTCCTGGA ACAACCTCAG AATGAACCGG GTACATTGCC AAATAATAGG 180  
ACATGACCCC TTAGTTACGT AGAATTCCTT TGGCAGAACC CCTTGTCGCT TGGCAGAACC 240  
CCTTAGTTAT GTAACTTGT ACTTTCCTG CCCGCTCTC CCCCTTGAG TTTTACTAT 300  
ATAAGC 306

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 305 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCTCCCATCT AGAGAGTGTT CCCAAAACAC TCCTGAACTC TTCACCCCAG AATGCATTCC 60  
 5 TGAACTCCTC ACCCTAAAGT TCAAACCCTC CCAACTAAAG ACTGTTCCAA GAACATTTTT 120  
 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCGGG TACATTGCCA AATAATAGGA 180  
 CATGACCCCT TAGTTACACA GAATTCCTT GGCAAAACCC CTTGTCCCTT GGCAGAACCC 240  
 10 CTTAGTTATG CAAACTTGTA CTTTCCCTGC CCAGCTCTCC CCCCTTGAGT TTTTCCTATA 300  
 TAAGC 305

## 15 (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 304 base pairs  
 (B) TYPE: nucleic acid  
 20 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

30 CCTCCCATCT AGAGAGTGTT CCCAGAACAC TCCTGAACTC TTCACCCCAG AATGCATTCC 60  
 TGAACTCCTC ACCCTAGAGT TTGAACCCTC CCAACTAAAG ACTGTTCCAA GAACATCTTT 120  
 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCGGG TACATTGCCA AATAATAGGA 180  
 35 CATGACCCCT TAGTTACGTA GAATTCCTT GGCAGAACCC CTTGTGCTT GGCAGAACCC 240  
 CTTAGTTATG CAAACTTGTA CTTTCCCTGC CCCGCTCTCC CCCTTGAGTT TTTCTATAT 300  
 40 AAGC 304

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH: 303 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 50 (ii) MOLECULE TYPE: DNA (genomic)

## 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCTCCCATCT AGAGAGTGTT CCCAGAACAC TCCTAAACTC TTCACCCCAG AATGCATTCC 60  
 TGAACTCCTC ACCCTAGAGT TCGAACCCTT CCAACTAAAG ACTGTTCCAA GAACATTTTT 120  
 60 GAGATAAGGG CCTCCTGGAA CAACCTCAAA ATGAACCGGG TACATTGCCA AATGATAGGA 180  
 CATGACCCCT TAGTTACGTA GATTCCTTG GCAGAACCC CTTGTCCCTTG GCAGAACCC 240  
 CTAGTGATGT AAACCTGTAC TTTCCCTGCC CAGCTCTCCC CCCTTGAGTT TTCCTATATA 300  
 65 AGC 303

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 8657 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

15 TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC 60  
CCCTCCCATC TGGAAAACAT ACTTGAGAAA AACATTTTCT GGAACAACCA CAGAATGTTT 120  
20 CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG 180  
TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCTTTA 240  
25 AAAGCTTGTG AAAAATTTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG 300  
AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTGCTGCT TTATTTGAC CCCAGAGCTC 360  
TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG 420  
30 GTCTCAGTGT CTTCTTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT 480  
TCCCTCGAGG GTCTTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG 540  
35 CATTGGCCGG GAATTCGAAA ATCTTTCATT TGGTGCAATTG GCCGGGAAAC AGCGCGACCA 600  
CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTTGT TCTGTTTTGG TCTGATGTCT 660  
GTGTTCTGAT GTCTGTGTTT TGTCTTAAG TCTGGTGCGA TCGCAGTTTC AGTTTTGCGG 720  
40 ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA 780  
GGTGTCCACC GTCCGTTTCG CCTGGGAGAC GTCCAGGAG GAACAGGGGA GGATCAGGGA 840  
45 CGCCTGGTGG ACCCCTTTGA AGGCCAAGAG ACCATTTGGG GTTGCGAGAT CGTGGGTTCG 900  
AGTCCCACCT CGTGCCAGT TGCAGATCG TGGGTTCGAG TCCCACCTCG TGTTTTGTTG 960  
CGAGATCGTG GGTTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC 1020  
50 ACCTCGTGTT TTGTTGCGAG ATCGTGGGTG CGAGTCCCAC CTCGCGTCTG GTCACGGGAT 1080  
CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT 1140  
55 CTGATTCTTC TGGTTTCTCT TTTTGTCTTA GTCTCGTGTG CGCTCTTGTT GTGACTACTG 1200  
TTTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTGCG 1260  
TTGGTAATTT TGTTTGTGTTA CGTTTGTGTTT TGTGAGTCGT CTATGTTGTC TGTTACTATC 1320  
60 TTGTTTTTGT TTGTGGTTTA CGGTTTCTGT GTGTGCTCTG TGTGTCTCTT TGTGTTTACA 1380  
CTTGGAATGA TGAATGACGA CTGTTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAAATC 1440  
65 CTGTCAGATC CCTATGCTGA CCACTTCCTT TCAGATCAAC AGCTGCCCTT ACGTATCGAT 1500

	GGATCCCTCG	ACTAACTAAT	AGCCCATTCT	CCAAGGTCGA	GCGGGATCAA	TTCCGCCCCC	1560
	CCCCTAACGT	TACTGGCCGA	AGCCGCTTGG	AATAAGGCCG	GTGTGCGTTT	GTCTATATGT	1620
5	TATTTTCCAC	CATATTGCCG	TCTTTTGGCA	ATGTGAGGGC	CCGGAACCT	GGCCCTGTCT	1680
	TCTTGACGAG	CATTCTAGG	GGTCTTTCCC	CTCTCGCCAA	AGGAATGCAA	GGTCTGTTGA	1740
10	ATGTCGTGAA	GGAAGCAGTT	CCTCTGGAAG	CTTCTTGAAG	ACAAACAACG	TCTGTAGCGA	1800
	CCCTTTGCAG	GCAGCGGAAC	CCCCACCTG	GCGACAGGTG	CCTCTGCGGC	CAAAAGCCAC	1860
	GTGTATAAGA	TACACCTGCA	AAGGCGGCAC	AACCCCAGTG	CCACGTTGTG	AGTTGGATAG	1920
15	TTGTGGAAAG	AGTCAAATGG	CTCTCCTCAA	GCGTATTCAA	CAAGGGGCTG	AAGGATGCCC	1980
	AGAAGGTACC	CCATTGTATG	GGATCTGATC	TGGGGCCTCG	GTGCACATGC	TTTACATGTG	2040
20	TTTAGTCGAG	GTTAAAAAAA	CGTCTAGGCC	CCCCGAACCA	CGGGGACGTG	GTTTTCTTTT	2100
	GAAAAACACG	ATAATAATCA	TGGGCGCGGA	TCCCGTCGTT	TTACAACGTC	GTGACTGGGA	2160
	AAACCCTGGC	GTTACCCAAC	TTAATCGCCT	TGCAGCACAT	CCCCCTTTCG	CCAGCTGGCG	2220
25	TAATAGCGAA	GAGGCCCGCA	CCGATCGCCC	TTCCAACAG	TTGCGCAGCC	TGAATGGCGA	2280
	ATGGCGCTTT	GCCTGGTTTC	CGGCACCAGA	AGCGGTGCCG	GAAAGCTGGC	TGGAGTGC GA	2340
30	TCTTCCTGAG	GCCGATACTG	TCGTCTGCCC	CTCAAAGTGG	CAGATGCACG	GTTACGATGC	2400
	GCCCATCTAC	ACCAACGTAA	CCTATCCCAT	TACGGTCAAT	CCGCCGTTTG	TTCCCACGGA	2460
	GAATCCGACG	GGTTGTTACT	CGCTCACATT	TAATGTTGAT	GAAAGCTGGC	TACAGGAAGG	2520
35	CCAGACGCGA	ATTATTTTTG	ATGGCGTTAA	CTCGGCGTTT	CATCTGTGGT	GCAACGGGCG	2580
	CTGGGTCTGGT	TACGGCCAGG	ACAGTCGTTT	GCCGTCTGAA	TTTGACCTGA	GCGCATTTTTT	2640
40	ACGCGCCGGA	GAAAACCGCC	TCGCGGTGAT	GGTGCTGCGT	TGGAGTGACG	GCAGTTATCT	2700
	GGAAGATCAG	GATATGTGGC	GGATGAGCGG	CATTTTCCGT	GACGTCTCGT	TGCTGCATAA	2760
	ACCGACTACA	CAAATCAGCG	ATTTCCATGT	TGCCACTCGC	TTTAATGATG	ATTTTACGCCG	2820
45	CGCTGTACTG	GAGGCTGAAG	TTCAGATGTG	CGGCGAGTTG	CGTGAATACC	TACGGGTAAC	2880
	AGTTTCTTTA	TGGCAGGGTG	AAACGCAGGT	CGCCAGCGGC	ACCGCGCCTT	TCGGCGGTGA	2940
50	AATTATCGAT	GAGCGTGGTG	GTTATGCCGA	TCGCGTCACA	CTACGTCTGA	ACGTGCAAAA	3000
	CCCGAAACTG	TGGAGCGCCG	AAATCCCGAA	TCTCTATCGT	GCGGTGGTTG	AACTGCACAC	3060
	CGCCGACGGC	ACGCTGATTG	AAGCAGAAGC	CTGCGATGTC	GGTTTCCGCG	AGGTGCGGAT	3120
55	TGAAAATGGT	CTGCTGCTGC	TGAACGGCAA	GCCGTTGCTG	ATTTCGAGCG	TTAACCGTCA	3180
	CGAGCATCAT	CCTCTGCATG	GTCAGGTCAT	GGATGAGCAG	ACGATGGTGC	AGGATATCCT	3240
	GCTGATGAAG	CAGAACAAC	TTAACGCCGT	GCGCTGTTTCG	CATTATCCGA	ACCATCCGCT	3300
60	GTGGTACACG	CTGTGCGACC	GCTACGGCCT	GTATGTGGTG	GATGAAGCCA	ATATTGAAAC	3360
	CCACGGCATG	GTGCCAATGA	ATCGTCTGAC	CGATGATCCG	CGCTGGCTAC	CGGCGATGAG	3420
65	CGAACGCGTA	ACGCGAATGG	TGCAGCGCGA	TCGTAATCAC	CCGAGTGTGA	TCATCTGGTC	3480
	GCTGGGGAAT	GAATCAGGCC	ACGGCGCTAA	TCACGACGCG	CTGTATCGCT	GGATCAAATC	3540



	TGTCGATCCT	TCCCGCCCGG	TGCAGTATGA	AGGCGGCGGA	GCCGACACCA	CGGCCACCGA	3600
	TATTATTTGC	CCGATGTACG	CGCGCGTGGA	TGAAGACCAG	CCCTTCCCGG	CTGTGCCGAA	3660
5	ATGGTCCATC	AAAAAATGGC	TTTCGCTACC	TGGAGAGACG	CGCCCCTGA	TCCTTTGCGA	3720
	ATACGCCCAC	GCGATGGGTA	ACAGTCTTGG	CGGTTTCGCT	AAATACTGGC	AGGCGTTTCG	3780
10	TCAGTATCCC	CGTTTACAGG	GCGGCTTCGT	CTGGGACTGG	GTGGATCAGT	CGCTGATTAA	3840
	ATATGATGAA	AACGGCAACC	CGTGGTCGGC	TTACGGCGGT	GATTTTGGCG	ATACGCCGAA	3900
	CGATCGCCAG	TTCTGTATGA	ACGGTCTGGT	CTTTGCCGAC	CGCACGCCGC	ATCCAGCGCT	3960
15	GACGGAAGCA	AAACACCAGC	AGCAGTTTTT	CCAGTTCCGT	TTATCCGGGC	AAACCATCGA	4020
	AGTGACCAGC	GAATACCTGT	TCCGTCATAG	CGATAACGAG	CTCCTGCACT	GGATGGTGGC	4080
20	GCTGGATGGT	AAGCCGCTGG	CAAGCGGTGA	AGTGCCTCTG	GATGTCGCTC	CACAAGGTAA	4140
	ACAGTTGATT	GAAGTGCCTG	AACTACCGCA	GCCGGAGAGC	GCCGGGCAAC	TCTGGCTCAC	4200
	AGTACGCGTA	GTGCAACCGA	ACGCGACCGC	ATGGTCAGAA	GCCGGGCACA	TCAGCGCCTG	4260
25	GCAGCAGTGG	CGTCTGGCGG	AAAACCTCAG	TGTGACGCTC	CCCGCCGCGT	CCCACGCCAT	4320
	CCCGCATCTG	ACCACCAGCG	AAATGGATTT	TTGCATCGAG	CTGGGTAATA	AGCGTTGGCA	4380
30	ATTTAACCGC	CAGTCAGGCT	TTCTTTCACA	GATGTGGATT	GGCGATAAAA	AACAAGTCT	4440
	GACGCCGCTG	CGCGATCAGT	TCACCCGTGC	ACCGCTGGAT	AACGACATTG	GCGTAAGTGA	4500
	AGCGACCCGC	ATTGACCCTA	ACGCCTGGGT	CGAACGCTGG	AAGGCGGCGG	GCCATTACCA	4560
35	GGCCGAAGCA	GCGTTGTTGC	AGTGCACGGC	AGATACACTT	GCTGATGCGG	TGCTGATTAC	4620
	GACCGCTCAC	GCGTGGCAGC	ATCAGGGGAA	AACCTTATTT	ATCAGCCGGA	AAACCTACCG	4680
40	GATTGATGGT	AGTGGTCAAA	TGGCGATTAC	CGTTGATGTT	GAAGTGGCGA	GCGATACACC	4740
	GCATCCGGCG	CGGATTGGCC	TGAACTGCCA	GCTGGCGCAG	GTAGCAGAGC	GGGTAAACTG	4800
	GCTCGGATTA	GGGCCGCAAG	AAAACATATC	CGACCGCCTT	ACTGCCGCCT	GTTTTGACCG	4860
45	CTGGGATCTG	CCATTGTCTAG	ACATGTATAC	CCCGTACGTC	TTCCCGAGCG	AAAACGGTCT	4920
	GCGCTGCGGG	ACGCGCGAAT	TGAATTATGG	CCCACACCAG	TGGCGCGGCG	ACTTCCAGTT	4980
50	CAACATCAGC	CGCTACAGTC	AACAGCAACT	GATGGAAACC	AGCCATCGCC	ATCTGCTGCA	5040
	CGCGGAAGAA	GGCACATGGC	TGAATATCGA	CGGTTTCCAT	ATGGGGATTG	GTGGCGACGA	5100
	CTCCTGGAGC	CCGTCAAGTAT	CGGCGGAATT	CCAGCTGAGC	GCCGGTCGCT	ACCATTACCA	5160
55	GTTGGTCTGG	TGTCAAAAAT	AATAATAACC	GGGCAGGGGG	GATCCGAAGG	CGGGGACAGC	5220
	AGTGCAGTGG	TGGACAGAAA	GCAAGTGATC	TAGGCCAGCA	GCCTCCCTAA	AGGGACTTCA	5280
	GCCCACAAAG	CCAAACTTGT	GGCTTTAATA	CAAGCTCTGT	AAATGGTAAA	AAAAAAAAAG	5340
60	TCTACACGGA	CAGCAGGTAT	GCTCTTGCCA	CTGTACAGAG	CAATATACAG	ACAAAGAGAA	5400
	CTGTTGACAT	CTGCAGAGAA	AGACCTAAGA	TGCTGTGGCT	AAAAGAAATC	AGATGGCAAA	5460
65	TCTAACCGCC	CAGGCATCCT	AAAGAGCAAT	GATCCTGACA	GTCTGAAGAC	TATCAAGTTA	5520

	TAGACAAATT	AAGACTGGTA	AAAAAAACCC	TGTATAAAAT	AGTAAAAACT	GAAAAAAGAA	5580
	AACTAGTCCT	CTCATGAGAA	GACAGACCTG	ACATCTACTG	AAAAATAGAC	TTTACTGGAA	5640
5	AAAATATGTG	TATGAATACC	TTCTAGTTTT	TGTGAACGTT	CTCAAGATGG	ATAAAAAGCTT	5700
	TTCCTTGTA	AACGAGACTG	ATCAGATAGT	CATCAAGAAG	ATTGTTAAAG	AAAATTTTCC	5760
	AAGGTTCCGA	GTGCCAAAAG	CAATAGTGTC	AGATAATGGT	CCTGCCTTTG	TTGCCCAGGT	5820
10	AAGTCAGGGT	GTGGCCAAGT	ATTTAGAGGT	CAAATGAAAA	TTCCATTGTG	TGTACAGACC	5880
	TCAGAGCTCA	GGAAAGATAA	AAAAGAATAA	ATAAACTCT	AAACAGACCT	TGACAAAATT	5940
15	AATCCTAGAG	ACTGGCACAG	ACTTACTTGG	TACTCCTTCC	CCTTGCCCTA	TTTAGAACTG	6000
	AGAATACTCC	CTCTTGATTG	GGTTTTACTC	TTTTTAAGAT	CCTTTATGGG	GCTCCTATGC	6060
	CATCACTGTC	TTAAATGATG	TGTTTAAACC	TATGTTGTGA	TAATAATGAT	CTATATGTGA	6120
20	AGTTAAAAGG	CTTGCAGGTG	GTGCAGAAAG	AAGTCTGGTC	ACAACTGGCT	ACAGTGAACA	6180
	AGCTGGGTAC	CCCAAGGACA	TCTTACCAGT	TCCAGCCAGA	GATCTGATCT	ACGATCCCCG	6240
25	GGTCGACCCG	GGTCGACCCT	GTGGAATGTG	TGTCAGTTAG	GGTGTGGAAA	GTCCCCAGGC	6300
	TCCCCAGCAG	GCAGAAGTAT	GCAAAGCATG	CATCTCAATT	AGTCAGCAAC	CAGGTGTGGA	6360
	AAGTCCCCAG	GCTCCCCAGC	AGGCAGAAAGT	ATGCAAAGCA	TGCATCTCAA	TTAGTCAGCA	6420
30	ACCATAGTCC	CGCCCCTAAC	TCCGCCCATC	CCGCCCTAA	CTCCGCCAG	TTCCGCCCAT	6480
	TCTCCGCCCC	ATGGCTGACT	AATTTTTTTT	ATTATGCAG	AGGCCGAGGC	CGCCTCGGCC	6540
35	TCTGAGCTAT	TCCAGAAGTA	GTGAGGAGGC	TTTTTTGGAG	GCCTAGGCTT	TTGCAAAAAG	6600
	CTTCACGCTG	CCGCAAGCAC	TCAGGGCGCA	AGGGCTGCTA	AAGGAAGCGG	AACACGTAGA	6660
	AAGCCAGTCC	GCAGAAACGG	TGCTGACCCC	GGATGAATGT	CAGCTACTGG	GCTATCTGGA	6720
40	CAAGGGAAAA	CGCAAGCGCA	AAGAGAAAGC	AGGTAGCTTG	CAGTGGGCTT	ACATGGCGAT	6780
	AGCTAGACTG	GGCGGTTTTA	TGGACAGCAA	GCGAACCGGA	ATTGCCAGCT	GGGGCGCCCT	6840
45	CTGGTAAGGT	TGGGAAGCCC	TGCAAAGTAA	ACTGGATGGC	TTTCTTGCCG	CCAAGGATCT	6900
	GATGGCGCAG	GGGATCAAGA	TCTGATCAAG	AGACAGGATG	AGGATCGTTT	CGCATGATTG	6960
	AACAAGATGG	ATTGCACGCA	GGTTCTCCGG	CCGCTTGGGT	GGAGAGGCTA	TTCGGCTATG	7020
50	ACTGGGCACA	ACAGACAATC	GGCTGCTCTG	ATGCCGCCGT	GTTCCGGCTG	TCAGCGCAGG	7080
	GGCGCCCGGT	TCTTTTTGTC	AAGACCGACC	TGTCCGGTGC	CCTGAATGAA	CTGCAGGACG	7140
55	AGGCAGCGCG	GCTATCGTGG	CTGGCCACGA	CGGGCGTTCC	TTGCGCAGCT	GTGCTCGACG	7200
	TTGTCACTGA	AGCGGGAAGG	GACTGGCTGC	TATTGGGCGA	AGTGCCGGGG	CAGGATCTCC	7260
	TGTCATCTCA	CCTTGCTCCT	GCCGAGAAAG	TATCCATCAT	GGCTGATGCA	ATGCGGCGGC	7320
60	TGCATACGCT	TGATCCGGCT	ACCTGCCCAT	TCGACCACCA	AGCGAAACAT	CGCATCGAGC	7380
	GAGCACGTAC	TCGGATGGAA	GCCGGTCTTG	TCGATCAGGA	TGATCTGGAC	GAAGAGCATC	7440
	AGGGGCTCGC	GCCAGCCGAA	CTGTTTCGCCA	GGCTCAAGGC	GCGCATGCCC	GACGGCGAGG	7500
65	ATCTCGTCGT	GACCCATGGC	GATGCCTGCT	TGCCGAATAT	CATGGTGGAA	AATGGCCGCT	7560

TTTCTGGATT CATCGACTGT GGCCGGCTGG GTGTGGCGGA CCGCTATCAG GACATAGCGT 7620  
 TGGCTACCCG TGATATTGCT GAAGAGCTTG GCGGCGAATG GGCTGACCGC TTCCTCGTGC 7680  
 5 TTTACGGTAT CGCCGCTCCC GATTTCGAGC GCATCGCCTT CTATCGCCTT CTTGACGAGT 7740  
 TCTTCTGAGC GGGACTCTGG GGTTTCGAAAT GACCGACCAA GCGACGCCCCA ACCTGCCATC 7800  
 10 ACGAGATTTT GATTCCACCG CCGCCTTCTA TGAAAGGTTG GGCTTCGGAA TCGTTTTCCG 7860  
 GGACGGAATT CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT 7920  
 GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC 7980  
 15 AGATACCAAA TACTGTCTTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG 8040  
 TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGCGG 8100  
 20 ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT 8160  
 CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGA GCGAACGACC TACACCGAAC 8220  
 TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG 8280  
 25 ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG 8340  
 GAAACGCCTG GSTATCTTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT GAGCGTCGAT 8400  
 30 TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCCGAGATGC 8460  
 GCCGCCTCGA GTACACCTGC GTCATGCTGA GACCCTCAAG CCTCACTAAA AGGGTCCCTG 8520  
 CCTAGTTCTG TTTACTAATC TGCCTTATTC TGTTTTTGTT CCCATGTTAA AGATAGAGTA 8580  
 35 AATGCAGTAT TCTCCACATA GAGATATAGA CTTCTGAAAT TCTAAGATTA GAATTATTTA 8640  
 CAAGAAGAAG TGGGGAA 8657

40 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6359 base pairs  
 (B) TYPE: nucleic acid  
 45 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

55 TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC 60  
 CCCTCCCATC TGGAACACAT ACTTGAGAAA AACATTTTCT GGAACAACCA CAGAATGTTT 120  
 CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG 180  
 60 TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCCTCCCA GTTTGTGGTT TTTTCTTTA 240  
 AAAGCTTGTG AAAAATTTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG 300  
 65 AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTTTCGAC CCCAGAGCTC 360

	TGGTCTGTGT	GCTTTCATGT	CGCTGCTTTA	TTAAATCTTA	CCTTCTACAT	TTTATGTATG	420
	GTCTCAGTGT	CTTCTTGGGT	ACGCGGCTGT	CCCGGGACTT	GAGTGTCTGA	GTGAGGGTCT	480
5	TCCCTCGAGG	GTCTTTCATT	TGGTACATGG	GCCGGGAATT	CGAGAATCTT	TCATTTGGTG	540
	CATTGGCCGG	GAATTCGAAA	ATCTTTCATT	TGGTGCATTG	GCCGGGAAAC	AGCGCGACCA	600
	CCCAGAGGTC	CTAGACCCAC	TTAGAGGTAA	GATTCTTTGT	TCTGTTTTGG	TCTGATGTCT	660
10	GTGTTCTGAT	GTCTGTGTTC	TGTTTCTAAG	TCTGGTGCGA	TCGCAGTTTC	AGTTTTGCGG	720
	ACGCTCAGTG	AGACCGCGCT	CCGAGAGGGA	GTGCGGGGTG	GATAAGGATA	GACGTGTCCA	780
15	GGTGTCCACC	GTCCGTTCGC	CCTGGGAGAC	GTCCCAGGAG	GAACAGGGGA	GGATCAGGGA	840
	CGCCTGGTGG	ACCCCTTTGA	AGGCCAAGAG	ACCATTGTTG	GTTGCGAGAT	CGTGGGTTCG	900
	AGTCCCACCT	CGTGCCCACT	TGCGAGATCG	TGGGTTTCAG	TCCCACCTCG	TGTTTTGTGT	960
20	CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	TCTGGTCACG	GGATCGTGGG	TTCGAGTCCC	1020
	ACCTCGTGTT	TTGTTGCGAG	ATCGTGGGTT	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	1080
25	CGTGGGTTCG	AGTCCCACCT	CGTGCAGAGG	GTCTCAATTG	GCCGGCCTTA	GAGAGGCCAT	1140
	CTGATTCTTC	TGGTTTCTCT	TTTTGTCTTA	GTCTCGTGTC	CGCTCTTGTT	GTGACTACTG	1200
	TTTTTCTAAA	AATGGGACAA	TCTGTGTCCA	CTCCCCTTTC	TCTGACTCTG	GTTCTGTTCG	1260
30	TTGGTAATTT	TGTTTGTTTA	CGTTTGTTTT	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	1320
	TTGTTTTTGT	TTGTGGTTTA	CGGTTTCTGT	GTGTGTCTTG	TGTGTCTCTT	TGTGTTTCTG	1380
35	CTTGGAATGA	TGACTGACGA	CTGTTTTTAA	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	1440
	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTT	ACTCGAGCTC	1500
	AAGCTTCGAA	TTCTGCAGTC	GACGGTACCG	CGGCCGCTAA	CTAATAGCCC	ATTCTCCAAG	1560
40	GTACGTAGCG	GGGATCAATT	CCGCCCCCCC	CCTAACGTTA	CTGGCCGAAG	CCGCTTGGA	1620
	TAAGGCCGGT	GTGCGTTTGT	CTATATGTTA	TTTTCCACCA	TATTGCCGTC	TTTTGGCAAT	1680
45	GTGAGGGCCC	GGAAACCTGG	CCCTGTCTTC	TTGACGAGCA	TTCCTAGGGG	TCTTTCCCCT	1740
	CTCGCCAAAG	GAATGCAAGG	TCTGTTGAAT	GTCGTGAAGG	AAGCAGTTCC	TCTGGAAGCT	1800
	TCTTGAAGAC	AAACAACGTC	TGTAGCGACC	CTTTGCAGGC	AGCGGAACCC	CCCACCTGGC	1860
50	GACAGGTGCC	TCTGCGGCCA	AAAGCCACGT	GTATAAGATA	CACCTGCAAA	GGCGGCACAA	1920
	CCCCAGTGCC	ACGTTGTGAG	TTGGATAGTT	GTGGAAAGAG	TCAAATGGCT	CTCCTCAAGC	1980
	GTATTCAACA	AGGGGCTGAA	GGATGCCCAG	AAGGTACCCC	ATTGTATGGG	ATCTGATCTG	2040
55	GGGCCTCGGT	GCACATGCTT	TACATGTGTT	TAGTCGAGGT	TAAAAAACG	TCTAGGCCCC	2100
	CCGAACCACG	GGGACGTGGT	TTTCCTTTGA	AAAACACGAT	ACGGGATCCA	CCGGTCGCCA	2160
60	CCATGGGTAA	AGGAGAAGAA	CTTTTCACAG	GAGTTGTCCC	AATTCTTGTT	GAATTAGATG	2220
	GTGATGTAA	TGGGCACAAA	TTTTCTGTCA	GTGGAGAGGG	TGAAGGTGAT	GCAACATACG	2280
	GAAACTTAC	CCTTAAATTT	ATTTGCACTA	CTGGAAACT	ACCTGTTCCA	TGGCCAACAC	2340
65	TTGTCACTAC	TTTCACTTAT	GGTGTTCAAT	GCTTTTCAAG	ATACCCAGAT	CATATGAAAC	2400

	GGCATGACTT	TTTCAAGAGT	GCCATGCCCC	AAGGTTATGT	ACAGGAAAGA	ACTATATTTT	2460
	TCAAAGATGA	CGGGAAC TAC	AAGACACGTG	CTGAAGTCAA	GTTTGAAGGT	GATACCCTTG	2520
5	TTAATAGAAT	CGAGTTAAAA	GGTATTGATT	TTAAAGAAGA	TGGAAACATT	CTTGGACACA	2580
	AATTGGAATA	CAACTATAAC	TCACACAATG	TATACATCAT	GGCAGACAAA	CAAAAGAATG	2640
10	GAACCAAAGT	TAAC TTCAA	ATTAGACACA	ACATTGAAGA	TGGAAGCGTT	CAACTAGCAG	2700
	ACCATTATCA	ACAAAATACT	CCAATTGGCG	ATGGCCCTGT	CCTTTTACCA	GACAACCATT	2760
	ACCTGTCCAC	ACAATCTGCC	CTTTTGAAAG	ATCCCAACGA	AAAGAGAGAC	CACATGGTCC	2820
15	TTCTTGAGTT	TGTAACAGCT	GCTGGGATTA	CACATGGCAT	GGATGAACTA	TACAAGTCCG	2880
	GATCTAGATA	ACTGTATCGA	TGGATCCGAA	GGCGGGGACA	GCAGTGCAGT	GGTGGACAGA	2940
20	AAGCAAGTGA	TCTAGGCCAG	CAGCCTCCCT	AAAGGGACTT	CAGCCCACAA	AGCCAAACTT	3000
	GTGGCTTTAA	TACAAGCTCT	GTAAATGGTA	AAAAAAAAAA	AGTCTACACG	GACAGCAGGT	3060
	ATGCTCTTGC	CACTGTACAG	AGCAATATAC	AGACAAAGAG	AACTGTTGAC	ATCTGCAGAG	3120
25	AAAGACCTAA	GATGCTGTGG	CTAAAAGAAA	TCAGATGGCA	AATCTAACCG	CCCAGGCATC	3180
	CTAAAGAGCA	ATGATCCTGA	CAGTCTGAAG	ACTATCAAGT	TATAGACAAA	TTAAGACTGG	3240
30	TAAAAAAAC	CCTGTATAAA	ATAGTAAAAA	CTGAAAAAAG	AAA ACTAGTC	CTCTCATGAG	3300
	AAGACAGACC	TGACATCTAC	TGAAAAATAG	ACTTTACTGG	AAAAAATATG	TGTATGAATA	3360
	CCTTCTAGTT	TTTGTGAACG	TTCTCAAGAT	GGATAAAAGC	TTTTCTTTGT	AAAACGAGAC	3420
35	TGATCAGATA	GTCATCAAGA	AGATTGT TAA	AGAAAATTTT	CCAAGGTTTCG	GAGTGCCAAA	3480
	AGCAATAGTG	TCAGATAATG	GTCCTGCCTT	TGTTGCC CAG	GTAAGTCAGG	GTGTGGCCAA	3540
40	GTATTTAGAG	GTCAAATGAA	AATTCCATTG	TGTGTACAGA	CCTCAGAGCT	CAGGAAAGAT	3600
	AAAAAAGAAT	AAATAAAACT	CTAAACAGAC	CTTGACAAAA	TTAATCCTAG	AGACTGGCAC	3660
	AGACTTACTT	GGTACTCCTT	CCCCTTGCCC	TATTTAGAAC	TGAGAATACT	CCCTCTTGAT	3720
45	TCGGTTTTAC	TCTTTTTTAAG	ATCCTTTTATG	GGGCTCCTAT	GCCATCACTG	TCTTAAATGA	3780
	TGTGTTTTAAA	CCTATGTTGT	TATAATAATG	ATCTATATGT	TAAGTTAAAA	GGCTTGCAGG	3840
50	TGGTGCAGAA	AGAAGTCTGG	TCACAACTGG	CTACAGTGAA	CAAGCTGGGT	ACCCCAAGGA	3900
	CATCTTACCA	GTTCCAGCCA	GAGATCTGAT	CTACGATCCC	CGGGTCGACC	CGGGTCGACC	3960
	CTGTGGAATG	TGTGTCAGTT	AGGGTGTGGA	AAGTCCCCAG	GCTCCCCAGC	AGGCAGAAGT	4020
55	ATGCAAAGCA	TGCATCTCAA	TTAGTCAGCA	ACCAGGTGTG	GAAAGTCCCC	AGGCTCCCCA	4080
	GCAGGCAGAA	GTATGCAAAG	CATGCATCTC	AATTAGTCAG	CAACCATAGT	CCCGCCCCTA	4140
	ACTCCGCCCA	TCCCGCCCCT	AACTCCGCCC	AGTTCCGCCC	ATTCTCCGCC	CCATGGCTGA	4200
60	CTAATTTTTT	TTATTTTATGC	AGAGGCCGAG	GCCGCCTCGG	CCTCTGAGCT	ATTCCAGAAG	4260
	TAGTGAGGAG	GCTTTTTTTGG	AGGCCTAGGC	TTTTGCAAAA	AGCTTCACGC	TGCCGCAAGC	4320
65	ACTCAGGGCG	CAAGGGCTGC	TAAAGGAAGC	GGAACACGTA	GAAAGCCAGT	CCGCAGAAAC	4380

	GGTGCTGACC	CCGGATGAAT	GTCAGCTACT	GGGCTATCTG	GACAAGGGAA	AACGCAAGCG	4440
	CAAAGAGAAA	GCAGGTAGCT	TGCAGTGGGC	TTACATGGCG	ATAGCTAGAC	TGGGCGGTTT	4500
5	TATGGACAGC	AAGCGAACCG	GAATTGCCAG	CTGGGGCGCC	CTCTGGTAAG	GTTGGGAAGC	4560
	CCTGCAAAGT	AAACTGGATG	GCTTTCTTGC	CGCCAAGGAT	CTGATGGCGC	AGGGGATCAA	4620
10	GATCTGATCA	AGAGACAGGA	TGAGGATCGT	TTCGCATGAT	TGAACAAGAT	GGATTGCACG	4680
	CAGGTTCTCC	GGCCGCTTGG	GTGGAGAGGC	TATTCGGCTA	TGACTGGGCA	CAACAGACAA	4740
	TCGGCTGCTC	TGATGCCGCC	GTGTTCCGGC	TGTCAGCGCA	GGGGCGCCCG	GTTCTTTTTG	4800
15	TCAAGACCGA	CCTGTCCGGT	GCCCTGAATG	AACTGCAGGA	CGAGGCAGCG	CGGCTATCGT	4860
	GGCTGGCCAC	GACGGGCGTT	CCTTGCGCAG	CTGTGCTCGA	CGTTGTCACT	GAAGCGGGAA	4920
20	GGGACTGGCT	GCTATTGGGC	GAAGTGCCGG	GGCAGGATCT	CCTGTCATCT	CACCTTGCTC	4980
	CTGCCGAGAA	AGTATCCATC	ATGGCTGATG	CAATGCGGCG	GCTGCATACG	CTTGATCCGG	5040
	CTACCTGCCC	ATTCGACCAC	CAAGCGAAAC	ATCGCATCGA	GCGAGCACGT	ACTCGGATGG	5100
25	AAGCCGGTCT	TGTCGATCAG	GATGATCTGG	ACGAAGAGCA	TCAGGGGCTC	GCGCCAGCCG	5160
	AACTGTTTCG	CAGGCTCAAG	GCGCGCATGC	CCGACGGCGA	GGATCTCGTC	GTGACCCATG	5220
30	GCGATGCCTG	CTTGCCGAAT	ATCATGGTGG	AAAATGGCCG	CTTTTCTGGA	TTCATCGACT	5280
	GTGGCCGGCT	GGGTGTGGCG	GACCGCTATC	AGGACATAGC	GTTGGCTACC	CGTGATATTG	5340
	CTGAAGAGCT	TGGCGGCGAA	TGGGCTGACC	GCTTCCTCGT	GCTTTACGGT	ATCGCCGCTC	5400
35	CCGATTCGCA	GCGCATCGCC	TTCTATCGCC	TTCTTGACGA	GTTCTTCTGA	GCGGGACTCT	5460
	GGGGTTCGAA	ATGACCGACC	AAGCGACGCC	CAACCTGCCA	TCACGAGATT	TCGATTCCAC	5520
40	CGCCGCCTTC	TATGAAAGGT	TGGGCTTCGG	AATCGTTTTT	CGGGACGGAA	TTCGTAATCT	5580
	GCTGCTTGCA	AACAAAAAAA	CCACCGCTAC	CAGCGGTGGT	TGTTTTGCCG	GATCAAGAGC	5640
	TACCAACTCT	TTTTCCGAAG	GTAAGTGGCT	TCAGCAGAGC	GCAGATACCA	AATACTGTCC	5700
45	TTCTAGTGTA	GCCGTAGTTA	GGCCACCACT	TCAAGAACTC	TGTAGCACCG	CCTACATACC	5760
	TCGCTCTGCT	AATCCTGTTA	CCAGTGGCTG	CTGCCAGTGG	CGATAAGTCG	TGTCTTACCG	5820
50	GGTTGGACTC	AAGACGATAG	TTACCGGATA	AGGCGCAGCG	GTCGGGCTGA	ACGGGGGGTT	5880
	CGTGACACAC	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	ACTGAGATAC	CTACAGCGTG	5940
	AGCATTGAGA	AAGCGCCACG	CTTCCCGAAG	GGAGAAAGGC	GGACAGGTAT	CCGGTAAGCG	6000
55	GCAGGGTCGG	AACAGGAGAG	CGCACGAGGG	AGCTTCCAGG	GGGAAACGCC	TGGTATCTTT	6060
	ATAGTCCTGT	CGGGTTTCGC	CACCTCTGAC	TTGAGCGTCG	ATTTTTGTGA	TGCTCGTCAG	6120
	GGGGGCGGAG	CCTATGGAAA	AACGCCAGCA	ACGCCGAGAT	GCGCCGCCTC	GAGTACACCT	6180
60	GCGTCATGCT	GAGACCCTCA	AGCCTCACTA	AAAGGGTCCC	TGCCTAGTTC	TGTTTACTAA	6240
	TCTGCCTTAT	TCTGTTTTTT	TTCCCATGTT	AAAGATAGAG	TAAATGCAGT	ATTCTCCACA	6300
65	TAGAGATATA	GACTTCTGAA	ATTCTAAGAT	TAGAATTATT	TACAAGAAGA	AGTGGGGAA	6359

(2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 6891 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

15 TGAAGAATAA AAAATTACTG GCCTCTTG TG AGAACATGAA CTTTCACCTC GGAGCCCACC 60  
CCCTCCCATC TGGAAAACAT ACTTGAGAAA AACATTTTCT GGAACAACCA CAGAATGTTT 120  
CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG 180  
20 TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCTTTA 240  
AAAGCTTG TG AAAAATTTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG 300  
25 AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTGCTGCT TTATTTTCGAC CCCAGAGCTC 360  
TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG 420  
GTCTCAGTGT CTTCTTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT 480  
30 TCCCTCGAGG GTCTTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG 540  
CATTGGCCGG GAATTCGAAA ATCTTTCATT TGGTGCAATG GCCGGGAAAC AGCGCGACCA 600  
35 CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTTGT TCTGTTTTGG TCTGATGTCT 660  
GTGTTCTGAT GTCTGTGTTC TGTTTCTAAG TCTGGTGCGA TCGCAGTTTC AGTTTTGCGG 720  
ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA 780  
40 GGTGTCCACC GTCCGTTCGC CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGA 840  
CGCCTGGTGG ACCCCTTTGA AGGCCAAGAG ACCATTTGGG GTTGCAGAT CGTGGGTTCG 900  
45 AGTCCCACCT CGTGCCAGT TCGAGATCG TGGGTTTCGAG TCCCACCTCG TGTTTTGTG 960  
CGAGATCGTG GGTTTCAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC 1020  
ACCTCGTGTT TTGTTGCGAG ATCGTGGGT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT 1080  
50 CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT 1140  
CTGATTCTTC TGGTTTCTCT TTTTGTCTTA GTCTCGTGTC CGCTCTTGTT GTGACTACTG 1200  
55 TTTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTGCG 1260  
TTGGTAATTT TGTTTGTGTTA CGTTTGTGTT TGTGAGTCGT CTATGTTGTC TGTTACTATC 1320  
TTGTTTTTGT TTGTGGTTTA CGGTTTCTGT GTGTGTCTTG TGTGTCTCTT TGTGTTTACA 1380  
60 CTTGGACTGA TGA CTGACTGACGA CTGTTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAATC 1440  
CTGTCAGATC CCTATGCTGA CCACTTCCTT TCAGATCAAC AGCTGCCCTT ACTCGAGCTC 1500  
65 AAGCTTCGAA TTCTGCAGTC GACGGTACCG CGGGGATCAA TTCCGCCCCC CCCCTAACGT 1560

	TACTGGCCGA	AGCCGCTTGG	AATAAGGCCG	GTGTGCGTTT	GTCTATATGT	TATTTTCCAC	1620
	CATATTGCCG	TCTTTTGGCA	ATGTGAGGGC	CCGAAACCT	GGCCCTGTCT	TCTTGACGAG	1680
5	CATTCTAGG	GGTCTTTCCC	CTCTCGCCAA	AGGAATGCAA	GGTCTGTTGA	ATGTCGTGAA	1740
	GGAAGCAGTT	CCTCTGGAAG	CTTCTTGAAG	ACAAACAACG	TCTGTAGCGA	CCCTTTGCAG	1800
10	GCAGCGGAAC	CCCCCACCTG	GCGACAGGTG	CCTCTGCGGC	CAAAGCCAC	GTGTATAAGA	1860
	TACACCTGCA	AAGGCGGCAC	AACCCCACTG	CCACGTTGTG	AGTTGGATAG	TTGTGGAAAG	1920
	AGTCAAATGG	CTCTCCTCAA	GCGTATTCAA	CAAGGGGCTG	AAGGATGCCC	AGAAGGTACC	1980
15	CCATTGTATG	GGATCTGATC	TGGGGCCTCG	GTGCACATGC	TTTACATGTG	TTTAGTCGAG	2040
	GTTAAAAAAC	GTCTAGGCC	CCCGAACCA	GGGGACGTGG	TTTTCTTTG	AAAAACACGA	2100
20	GCGGGATCAA	TTCCGCCCC	CCCCTAACGT	TACTGGCCGA	AGCCGCTTGG	AATAAGGCCG	2160
	GTGTGCGTTT	GTCTATATGT	TATTTTCCAC	CATATTGCCG	TCTTTTGGCA	ATGTGAGGGC	2220
	CCGGAAACCT	GGCCCTGTCT	TCTTGACGAG	CATTCTAGG	GGTCTTTCCC	CTCTCGCCAA	2280
25	AGGAATGCAA	GGTCTGTTGA	ATGTCGTGAA	GGAAGCAGTT	CCTCTGGAAG	CTTCTTGAAG	2340
	ACAAACAACG	TCTGTAGCGA	CCCTTTGCAG	GCAGCGGAAC	CCCCCACCTG	GCGACAGGTG	2400
30	CCTCTGCGGC	CAAAGCCAC	GTGTATAAGA	TACACCTGCA	AAGGCGGCAC	AACCCCACTG	2460
	CCACGTTGTG	AGTTGGATAG	TTGTGGAAAG	AGTCAAATGG	CTCTCCTCAA	GCGTATTCAA	2520
	CAAGGGGCTG	AAGGATGCCC	AGAAGGTACC	CCATTGTATG	GGATCTGATC	TGGGGCCTCG	2580
35	GTGCACATGC	TTTACATGTG	TTTAGTCGAG	GTTAAAAAA	CGTCTAGGCC	CCCGAACCA	2640
	CGGGGACGTG	GTTTTCTTT	GAAAAACACG	ATACGGGATC	CACCGGTCGC	CACCATGGGT	2700
40	AAAGGAGAAG	AACTTTTCAC	AGGAGTTGTC	CCAATTCTTG	TTGAATTAGA	TGGTGATGTT	2760
	AATGGGCACA	AATTTTCTGT	CAGTGGAGAG	GGTGAAGGTG	ATGCAACATA	CGGAAAACCT	2820
	ACCCTTAAAT	TTATTTGCAC	TACTGGAAAA	CTACCTGTTC	CATGGCCAAC	ACTTGTCACT	2880
45	ACTTTCACCT	ATGGTGTTCA	ATGCTTTTCA	AGATACCCAG	ATCATATGAA	ACGGCATGAC	2940
	TTTTTCAAGA	GTGCCATGCC	CGAAGGTTAT	GTACAGGAAA	GAATATATT	TTTCAAAGAT	3000
	GACGGGAAC	ACAAGACACG	TGCTGAAGTC	AAGTTTGAAG	GTGATACCT	TGTTAATAGA	3060
50	ATCGAGTTAA	AAGGTATTGA	TTTTAAAGAA	GATGGAAACA	TTCTTGGACA	CAAATTGGAA	3120
	TACAACATA	ACTCACACAA	TGTATACATC	ATGGCAGACA	AACAAAAGAA	TGGAACCAAA	3180
55	GTAACTTCA	AAATTAGACA	CAACATTGAA	GATGGAAGCG	TTCAACTAGC	AGACCATTAT	3240
	CAACAAATA	CTCCAATTGG	CGATGGCCCT	GTCCTTTTAC	CAGACAACCA	TTACCTGTCC	3300
	ACACAATCTG	CCCTTTCGAA	AGATCCCAAC	GAAAAGAGAG	ACCACATGGT	CCTTCTTGAG	3360
60	TTTGTAACAG	CTGCTGGGAT	TACACATGGC	ATGGATGAAC	TATACAAGTC	CGGATCTAGA	3420
	TAACTGTATC	GATGGATCCG	AAGGCGGGGA	CAGCAGTGCA	GTGGTGGACA	GAAAGCAAGT	3480
65	GATCTAGGCC	AGCAGCCTCC	CTAAAGGGAC	TTCAGCCCAC	AAAGCCAAAC	TTGTGGCTTT	3540
	AATACAAGCT	CTGTAAATGG	TAAAAAATA	AAAGTCTACA	CGGACAGCAG	GTATGCTCTT	3600



	GCCACTGTAC	AGAGCAATAT	ACAGACAAAG	AGAACTGTTG	ACATCTGCAG	AGAAAGACCT	3660
	AAGATGCTGT	GGCTAAAAGA	AATCAGATGG	CAAATCTAAC	CGCCCAGGCA	TCCTAAAGAG	3720
5	CAATGATCCT	GACAGTCTGA	AGACTATCAA	GTTATAGACA	AATTAAGACT	GGTAAAAAAA	3780
	ACCCTGTATA	AAATAGTAAA	AACTGAAAAA	AGAAAACTAG	TCCTCTCATG	AGAAGACAGA	3840
10	CCTGACATCT	ACTGAAAAAT	AGACTTTACT	GGAAAAAATA	TGTGTATGAA	TACCTTCTAG	3900
	TTTTTGTGAA	CGTTCTCAAG	ATGGATAAAA	GCTTTTCCTT	GTAAAACGAG	ACTGATCAGA	3960
	TAGTCATCAA	GAAGATTGTT	AAAGAAAAAT	TTCCAAGGTT	CGGAGTGCCA	AAAGCAATAG	4020
15	TGTCAGATAA	TGGTCTGCC	TTTGTGCCC	AGGTAAGTCA	GGGTGTGGCC	AAGTATTTAG	4080
	AGGTCAAATG	AAAATTCCAT	TGTGTGTACA	GACCTCAGAG	CTCAGGAAAG	ATAAAAAAGA	4140
20	ATAAATAAAA	CTCTAAACAG	ACCTTGACAA	AATTAATCCT	AGAGACTGGC	ACAGACTTAC	4200
	TTGGTACTCC	TTCCCCCTGC	CCTATTTAGA	ACTGAGAATA	CTCCCTCTTG	ATTCGGTTTT	4260
	ACTCTTTTTA	AGATCCTTTA	TGGGGCTCCT	ATGCCATCAC	TGTCTTAAAT	GATGTGTTTA	4320
25	AACCTATGTT	GTTATAATAA	TGATCTATAT	GTTAAGTTAA	AAGGCTTGCA	GGTGGTGCG	4380
	AAAGAAGTCT	GGTCACAAC	GGCTACAGTG	AACAAGCTGG	GTACCCCAAG	GACATCTTAC	4440
30	CAGTTCCAGC	CAGAGATCTG	ATCTACGATC	CCCGGGTCGA	CCCGGGTCGA	CCCTGTGGAA	4500
	TGTGTGTCAG	TTAGGGTGTG	GAAAGTCCCC	AGGCTCCCCA	GCAGGCAGAA	GTATGCAAAG	4560
	CATGCATCTC	AATTAGTCAG	CAACCAGGTG	TGGAAAGTCC	CCAGGCTCCC	CAGCAGGCAG	4620
35	AAGTATGCAA	AGCATGCATC	TCAATTAGTC	AGCAACCATA	GTCCCGCCCC	TAACCTCCGC	4680
	CATCCCGCCC	CTAACTCCGC	CCAGTTCCGC	CCATTCTCCG	CCCCATGGCT	GAATAATTTT	4740
40	TTTTATTTAT	GCAGAGGCCG	AGGCCGCTC	GGCCTCTGAG	CTATTCCAGA	AGTAGTGAGG	4800
	AGGCTTTTTT	GGAGGCCTAG	GCTTTTGCAA	AAAGCTTCAC	GCTGCCGCAA	GCACTCAGGG	4860
	CGCAAGGGCT	GCTAAAGGAA	GCGGAACACG	TAGAAAGCCA	GTCCGCAGAA	ACGGTGCTGA	4920
45	CCCCGGATGA	ATGTCAGCTA	CTGGGCTATC	TGGACAAGGG	AAAACGCAAG	CGCAAAGAGA	4980
	AAGCAGGTAG	CTTGCACTGG	GCTTACATGG	CGATAGCTAG	ACTGGGCGGT	TTTATGGACA	5040
	GCAAGCGAAC	CGGAATTGCC	AGCTGGGGCG	CCCTCTGGTA	AGGTTGGGAA	GCCCTGCAAA	5100
50	GTAAACTGGA	TGGCTTTCTT	GCCGCCAAGG	ATCTGATGGC	GCAGGGGATC	AAGATCTGAT	5160
	CAAGAGACAG	GATGAGGATC	GTTTCGCATG	ATTGAACAAG	ATGGATTGCA	CGCAGGTTCT	5220
55	CCGGCCGCTT	GGGTGGAGAG	GCTATTCGGC	TATGACTGGG	CACAACAGAC	AATCGGCTGC	5280
	TCTGATGCCG	CCGTGTTCCG	GCTGTCAGCG	CAGGGGCGCC	CGGTTCTTTT	TGTCAAGACC	5340
	GACCTGTCCG	GTGCCCTGAA	TGAACTGCAG	GACGAGGCAG	CGCGGCTATC	GTGGCTGGCC	5400
60	ACGACGGGCG	TTCCTTGCGC	AGCTGTGCTC	GACGTTGTCA	CTGAAGCGGG	AAGGGACTGG	5460
	CTGCTATTGG	GCGAAGTGCC	GGGGCAGGAT	CTCCTGTCAT	CTCACCTTGC	TCCTGCCGAG	5520
65	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG	CGGCTGCATA	CGCTTGATCC	GGCTACCTGC	5580

	CCATTCGACC	ACCAAGCGAA	ACATCGCATC	GAGCGAGCAC	GTACTCGGAT	GGAAGCCGGT	5640
	CTTGTCGATC	AGGATGATCT	GGACGAAGAG	CATCAGGGGC	TCGCGCCAGC	CGAACTGTTC	5700
5	GCCAGGCTCA	AGGCGCGCAT	GCCCGACGGC	GAGGATCTCG	TCGTGACCCA	TGGCGATGCC	5760
	TGCTTGCCGA	ATATCATGGT	GGAAAATGGC	CGCTTTTCTG	GATTCATCGA	CTGTGGCCGG	5820
	CTGGGTGTGG	CGGACCGCTA	TCAGGACATA	GCGTTGGCTA	CCCGTGATAT	TGCTGAAGAG	5880
10	CTTGCGGGCG	AATGGGCTGA	CCGCTTCCTC	GTGCTTTACG	GTATCGCCGC	TCCCGATTCTG	5940
	CAGCGCATCG	CCTTCTATCG	CCTTCTTGAC	GAGTTCTTCT	GAGCGGGACT	CTGGGGTTCTG	6000
15	AAATGACCGA	CCAAGCGACG	CCCAACCTGC	CATCACGAGA	TTTCGATTCC	ACCGCCGCCT	6060
	TCTATGAAAG	GTTGGGCTTC	GGAATCGTTT	TCCGGGACGG	AATTCGTAAT	CTGCTGCTTG	6120
	CAAACAAAA	AACCACCGCT	ACCAGCGGTG	GTTTGTTCG	CGGATCAAGA	GCTACCAACT	6180
20	CTTTTTCCGA	AGGTAAGTGG	CTTCAGCAGA	GCGCAGATAC	CAAATACTGT	CCTTCTAGTG	6240
	TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	TCTGTAGCAC	CGCCTACATA	CCTCGCTCTG	6300
25	CTAATCCTGT	TACCAGTGGC	TGCTGCCAGT	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	6360
	TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	CGGTCGGGCT	GAACGGGGGG	TTCGTGCACA	6420
	CAGCCCAGCT	TGGAGCGAAC	GACCTACACC	GAAGTGAAGT	ACCTACAGCG	TGAGCATTGA	6480
30	GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG	GCGGACAGGT	ATCCGGTAAG	CGGCAGGGTC	6540
	GGAACAGGAG	AGCGCACGAG	GGAGCTTCCA	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	6600
35	GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT	CGATTTTTGT	GATGCTCGTC	AGGGGGGCGG	6660
	AGCCTATGGA	AAAACGCCAG	CAACGCCGAG	ATGCGCCGCC	TCGAGTACAC	CTGCGTCATG	6720
	CTGAGACCCT	CAAGCCTCAC	TAAAAGGGTC	CCTGCCTAGT	TCTGTTTACT	AATCTGCCTT	6780
40	ATTCTGTTTT	TGTTCCCATG	TTAAAGATAG	AGTAAATGCA	GTATTCTCCA	CATAGAGATA	6840
	TAGACTTCTG	AAATTCTAAG	ATTAGAATTA	TTTACAAGAA	GAAGTGGGGA	A	6891
45	(2) INFORMATION FOR SEQ ID NO:17:						
	(i) SEQUENCE CHARACTERISTICS:						
	(A) LENGTH: 6321 base pairs						
	(B) TYPE: nucleic acid						
	(C) STRANDEDNESS: single						
50	(D) TOPOLOGY: linear						
	(ii) MOLECULE TYPE: DNA (genomic)						
55							
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:						
	TGAAGAATAA	AAAATTACTG	GCCTCTTGTG	AGAACATGAA	CTTTCACCTC	GGAGCCCACC	60
60	CCCTCCCATC	TGGAAAACAT	ACTTGAGAAA	AACATTTTCT	GGAACAACCA	CAGAATGTTT	120
	CAACAGGCCA	GATGTATTGC	CAAACACAGG	ATATGACTCT	TTGGTTGAGT	AAATTTGTGG	180
65	TTGTTAAACT	TCCCCTATTC	CCTCCCCATT	CCCCCTCCCA	GTTTGTGGTT	TTTTCCTTTA	240

	AAAGCTTGTG	AAAAATTTGA	GTCGTCGTCG	AGACTCCTCT	ACCCTGTGCA	AAGGTGTATG	300
	AGTTTCGACC	CCAGAGCTCT	GTGTGCTTTC	TGTTGCTGCT	TTATTTTCGAC	CCCAGAGCTC	360
5	TGGTCTGTGT	GCTTTCATGT	CGCTGCTTTA	TTAAATCTTA	CCTTCTACAT	TTTATGTATG	420
	GTCTCAGTGT	CTTCTTGGGT	ACGCGGCTGT	CCCGGGACTT	GAGTGTCTGA	GTGAGGGTCT	480
10	TCCCTCGAGG	GTCTTTTCATT	TGGTACATGG	GCCGGGAATT	CGAGAATCTT	TCATTTGGTG	540
	CATTGGCCGG	GAATTCGAAA	ATCTTTCATT	TGGTGCAATG	GCCGGGAAAC	AGCGCGACCA	600
	CCCAGAGGTC	CTAGACCCAC	TTAGAGGTAA	GATTCTTTGT	TCTGTTTTGG	TCTGATGTCT	660
15	GTGTTCTGAT	GTCTGTGTTC	TGTTTCTAAG	TCTGGTGCGA	TCGCAGTTTC	AGTTTTGCGG	720
	ACGCTCAGTG	AGACCGCGCT	CCGAGAGGGA	GTGCGGGGTG	GATAAGGATA	GACGTGTCCA	780
20	GGTGTCCACC	GTCCGTTTCG	CCTGGGAGAC	GTCCCAGGAG	GAACAGGGGA	GGATCAGGGA	840
	CGCCTGGTGG	ACCCCTTTGA	AGGCCAAGAG	ACCATTTGGG	GTTGCGAGAT	CGTGGGTTCG	900
	AGTCCCACCT	CGTGCCCACT	TGCGAGATCG	TGGGTTTCAG	TCCCACCTCG	TGTTTTGTTG	960
25	CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	TCTGGTCACG	GGATCGTGGG	TTGCAGTCCC	1020
	ACCTCGTGTT	TTGTTGCGAG	ATCGTGGGTT	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	1080
30	CGTGGGTTCG	AGTCCCACCT	CGTGCGAGAG	GTCTCAATTG	GCCGGCCTTA	GAGAGGCCAT	1140
	CTGATTCTTC	TGGTTTCTCT	TTTTGTCTTA	GTCTCGTGTC	CGCTCTTGTT	GTGACTACTG	1200
	TTTTTCTAAA	AATGGGACAA	TCTGTGTCCA	CTCCCCTTTC	TCTGACTCTG	GTTCTGTTCG	1260
35	TTGGTAATTT	TGTTTGTTTA	CGTTTGTTTT	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	1320
	TTGTTTTTGT	TTGTGGTTTA	CGGTTTCTGT	GTGTGTCTTG	TGTGTCTCTT	TGTGTTTCTG	1380
40	CTTGGAATGA	TGACTGACGA	CTGTTTTTAA	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	1440
	CTGTGAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTT	ACTCGAGCTC	1500
	AAGCTTCGAA	TTCTGCAGTC	GACGGTACCG	CGGGGATCAA	TTCCGCCCCC	CCCCTAACGT	1560
45	TACTGGCCGA	AGCCGCTTGG	AATAAGGCCG	GTGTGCGTTT	GTCTATATGT	TATTTTCCAC	1620
	CATATTGCCG	TCTTTTGGCA	ATGTGAGGGC	CCGGAAACCT	GGCCCTGTCT	TCTTGACGAG	1680
50	CATTCCTAGG	GGTCTTTCCC	CTCTCGCCAA	AGGAATGCAA	GGTCTGTTGA	ATGTCGTGAA	1740
	GGAAGCAGTT	CCTCTGGAAG	CTTCTTGAAG	ACAAACAACG	TCTGTAGCGA	CCCTTTGCAG	1800
	GCAGCGGAAC	CCCCACCTG	GCGACAGGTG	CCTCTGCGGC	CAAAAGCCAC	GTGTATAAGA	1860
55	TACACCTGCA	AAGGCGGCAC	AACCCCACTG	CCACGTTGTG	AGTTGGATAG	TTGTGGAAAG	1920
	AGTCAAATGG	CTCTCCTCAA	GCGTATTCAA	CAAGGGGCTG	AAGGATGCCC	AGAAGGTACC	1980
60	CCATTGTATG	GGATCTGATC	TGGGGCCTCG	GTGCACATGC	TTTACATGTG	TTTAGTCGAG	2040
	GTAAAAAAA	CGTCTAGGCC	CCCCGAACCA	CGGGGACGTG	GTTTTCTTTT	GAAAAACACG	2100
	ATACGGGATC	CACCGGTCGC	CACCATGGGT	AAAGGAGAAG	AACTTTTCAC	AGGAGTTGTC	2160
65	CCAATTCTTG	TTGAATTAGA	TGGTGATGTT	AATGGGCACA	AATTTTCTGT	CAGTGGAGAG	2220

	GGTGAAGGTG	ATGCAACATA	CGGAAAACTT	ACCCTTAAAT	TTATTTGCAC	TACTGGAAAA	2280
	CTACCTGTTC	CATGGCCAAC	ACTTGTCACT	ACTTTCACCT	ATGGTGTTCA	ATGCTTTTCA	2340
5	AGATACCCAG	ATCATATGAA	ACGGCATGAC	TTTTTCAAGA	GTGCCATGCC	CGAAGGTTAT	2400
	GTACAGGAAA	GAACATATAT	TTTCAAAGAT	GACGGGAACT	ACAAGACACG	TGCTGAAGTC	2460
10	AAGTTTGAAG	GTGATACCCT	TGTTAATAGA	ATCGAGTTAA	AAGGTATTGA	TTTTAAAGAA	2520
	GATGGAAACA	TTCTTGGAACA	CAAATTGGAA	TACAACTATA	ACTCACACAA	TGTATACATC	2580
	ATGGCAGACA	AACAAAAGAA	TGGAACCAAA	GTAACTTCA	AAATTAGACA	CAACATTGAA	2640
15	GATGGAAGCG	TTCAACTAGC	AGACCATTAT	CAACAAAATA	CTCCAATTGG	CGATGGCCCT	2700
	GTCCTTTTAC	CAGACAACCA	TTACCTGTCC	ACACAATCTG	CCCTTTCGAA	AGATCCCAAC	2760
20	GAAAAGAGAG	ACCACATGGT	CCTTCTTGAG	TTTGTAACAG	CTGCTGGGAT	TACACATGGC	2820
	ATGGATGAAC	TATACAAGTC	CGGATCTAGA	TAAGTGTATC	GATGGATCCG	AAGGCGGGGA	2880
	CAGCAGTGCA	GTGGTGGACA	GAAAGCAAGT	GATCTAGGCC	AGCAGCCTCC	CTAAAGGGAC	2940
25	TTCAGCCAC	AAAGCCAAAC	TTGTGGCTTT	AATACAAGCT	CTGTAAATGG	TAAAAAAAAA	3000
	AAAGTCTACA	CGGACAGCAG	GTATGCTCTT	GCCACTGTAC	AGAGCAATAT	ACAGACAAAG	3060
30	AGAAGTGTG	ACATCTGCAG	AGAAAGACCT	AAGATGCTGT	GGCTAAAAGA	AATCAGATGG	3120
	CAAATCTAAC	CGCCAGGCA	TCCTAAAGAG	CAATGATCCT	GACAGTCTGA	AGACTATCAA	3180
	GTTATAGACA	AATTAAGACT	GGTAAAAAAA	ACCCTGTATA	AAATAGTAAA	AACTGAAAAA	3240
35	AGAAAAGTAG	TCCTCTCATG	AGAAGACAGA	CCTGACATCT	ACTGAAAAAT	AGACTTTACT	3300
	GGAAAAAATA	TGTGTATGAA	TACCTTCTAG	TTTTTGTGAA	CGTTCTCAAG	ATGGATAAAA	3360
40	GCTTTTCCTT	GTAAAACGAG	ACTGATCAGA	TAGTCATCAA	GAAGATTGTT	AAAGAAAATT	3420
	TTCCAAGGTT	CGGAGTGCCA	AAAGCAATAG	TGTCAGATAA	TGGTCCTGCC	TTTGTGTGCC	3480
45	AGGTAAGTCA	GGGTGTGGCC	AAGTATTTAG	AGGTCAAATG	AAAATTCCAT	TGTGTGTACA	3540
	GACCTCAGAG	CTCAGGAAAG	ATAAAAAAGA	ATAAATAAAA	CTCTAAACAG	ACCTTGACAA	3600
	AATTAATCCT	AGAGACTGGC	ACAGACTTAC	TTGGTACTCC	TTCCCCTTGC	CCTATTTAGA	3660
	ACTGAGAATA	CTCCCTCTTG	ATTTCGGTTTT	ACTCTTTTTA	AGATCCTTTA	TGGGGCTCCT	3720
50	ATGCCATCAC	TGTCTTAAAT	GATGTGTTTA	AACCTATGTT	GTTATAATAA	TGATCTATAT	3780
	GTTAAGTTAA	AAGGCTTGCA	GGTGGTGCAG	AAAGAAGTCT	GGTCACAACT	GGCTACAGTG	3840
55	AACAAGCTGG	GTACCCCAAG	GACATCTTAC	CAGTTCCAGC	CAGAGATCTG	ATCTACGATC	3900
	CCCGGGTCGA	CCCGGGTCGA	CCCTGTGGAA	TGTGTGTCAG	TAGGGTGTG	GAAAGTCCCC	3960
	AGGCTCCCCA	GCAGGCAGAA	GTATGCAAAG	CATGCATCTC	AATTAGTCAG	CAACCAGGTG	4020
60	TGGAAAGTCC	CCAGGCTCCC	CAGCAGGCAG	AAGTATGCAA	AGCATGCATC	TCAATTAGTC	4080
	AGCAACCATA	GTCCCGCCCC	TAAGTCCGCC	CATCCCGCCC	CTAACTCCGC	CCAGTTCCGC	4140
65	CCATTCTCCG	CCCCATGGCT	GACTAATTTT	TTTTATTTAT	GCAGAGGCCG	AGGCCGCCTC	4200
	GGCCTCTGAG	CTATTCCAGA	AGTAGTGAGG	AGGCTTTTTT	GGAGGCCTAG	GCTTTTGCAA	4260

	AAAGCTTCAC	GCTGCCGCAA	GCACTCAGGG	CGCAAGGGCT	GCTAAAGGAA	GCGGAACACG	4320
	TAGAAAGCCA	GTCCGCAGAA	ACGGTGCTGA	CCCCGGATGA	ATGTCAGCTA	CTGGGCTATC	4380
5	TGGACAAGGG	AAAACGCAAG	CGCAAAGAGA	AAGCAGGTAG	CTTGCACTGG	GCTTACATGG	4440
	CGATAGCTAG	ACTGGGCGGT	TTTATGGACA	GCAAGCGAAC	CGGAATTGCC	AGCTGGGGCG	4500
10	CCCTCTGGTA	AGGTTGGGAA	GCCCTGCAAA	GTAAACTGGA	TGGCTTTCTT	GCCGCCAAGG	4560
	ATCTGATGGC	GCAGGGGATC	AAGATCTGAT	CAAGAGACAG	GATGAGGATC	GTTTCGCATG	4620
	ATTGAACAAG	ATGGATTGCA	CGCAGGTTCT	CCGGCCGCTT	GGGTGGAGAG	GCTATTCCGC	4680
15	TATGACTGGG	CACAACAGAC	AATCGGCTGC	TCTGATGCCG	CCGTGTTCCG	GCTGTCAGCG	4740
	CAGGGGCGCC	CGGTTCTTTT	TGTCAAGACC	GACCTGTCCG	GTGCCCTGAA	TGAACTGCAG	4800
20	GACGAGGCAG	CGCGGCTATC	GTGGCTGGCC	ACGACGGGCG	TTCCTTGCGC	AGCTGTGCTC	4860
	GACGTTGTCA	CTGAAGCGGG	AAGGGACTGG	CTGCTATTGG	GCGAAGTGCC	GGGGCAGGAT	4920
	CTCCTGTCAT	CTCACCTTGC	TCCTGCCGAG	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG	4980
25	CGGCTGCATA	CGCTTGATCC	GGCTACCTGC	CCATTCGACC	ACCAAGCGAA	ACATCGCATC	5040
	GAGCGAGCAC	GTACTCGGAT	GGAAGCCGGT	CTTGTCGATC	AGGATGATCT	GGACGAAGAG	5100
30	CATCAGGGGC	TCGCGCCAGC	CGAACTGTTT	GCCAGGCTCA	AGGCGCGCAT	GCCCGACGGC	5160
	GAGGATCTCG	TCGTGACCCA	TGGCGATGCC	TGCTTGCCGA	ATATCATGGT	GGAAAATGGC	5220
	CGCTTTTCTG	GATTCATCGA	CTGTGGCCGG	CTGGGTGTGG	CGGACCGCTA	TCAGGACATA	5280
35	GCGTTGGCTA	CCCGTGATAT	TGCTGAAGAG	CTTGGCGGCG	AATGGGCTGA	CCGCTTCCTC	5340
	GTGCTTTACG	GTATCGCCGC	TCCCGATTCT	CAGCGCATCG	CCTTCTATCG	CCTTCTTGAC	5400
40	GAGTTCTTCT	GAGCGGGACT	CTGGGGTTCT	AAATGACCGA	CCAAGCGACG	CCCAACCTGC	5460
	CATCACGAGA	TTTCGATTCC	ACCGCCGCCT	TCTATGAAAG	GTTGGGCTTC	GGAATCGTTT	5520
	TCCGGGACGG	AATTCGTAAT	CTGCTGCTTG	CAAACAAAAA	AACCACCGCT	ACCAGCGGTG	5580
45	GTTTGTTTGC	CGGATCAAGA	GCTACCAACT	CTTTTTCCGA	AGGTAAGTGG	CTTCAGCAGA	5640
	GCGCAGATAC	CAAATACTGT	CCTTCTAGTG	TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	5700
	TCTGTAGCAC	CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	TGCTGCCAGT	5760
50	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	5820
	CGGTCGGGCT	GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	GACCTACACC	5880
55	GAACTGAGAT	ACCTACAGCG	TGAGCATTGA	GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG	5940
	GCGGACAGGT	ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	GGAGCTTCCA	6000
	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	GTCGGGTTC	GCCACCTCTG	ACTTGAGCGT	6060
60	CGATTTTTGT	GATGCTCGTC	AGGGGGGCGG	AGCCTATGGA	AAAACGCCAG	CAACGCCGAG	6120
	ATGCGCCGCC	TCGAGTACAC	CTGCGTCATG	CTGAGACCCT	CAAGCCTCAC	TAAAAGGGTC	6180
65	CCTGCCTAGT	TCTGTTTACT	AATCTGCCTT	ATTCTGTTTT	TGTTCCCATG	TTAAAGATAG	6240

AGTAAATGCA GTATTCTCCA CATAGAGATA TAGACTTCTG AAATTCTAAG ATTAGAATTA 6300  
 TTTACAAGAA GAAGTGGGGA A 6321

5 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5754 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

20 TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC 60  
 CCCTCCCATC TGGAAAACAT ACTTGAGAAA AACATTTTCT GGAACAACCA CAGAATGTTT 120  
 CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG 180  
 25 TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCTTTA 240  
 AAAGCTTGTG AAAAATTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG 300  
 30 AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTGCTGCT TTATTTTCGAC CCCAGAGCTC 360  
 TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CTTTCTACAT TTTATGTATG 420  
 GTCTCAGTGT CTTCTTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT 480  
 35 TCCCTCGAGG GTCTTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG 540  
 CATTGGCCGG GAATTCGAAA ATCTTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA 600  
 40 CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTTGT TCTGTTTTGG TCTGATGTCT 660  
 GTGTTCTGAT GTCTGTGTTT TGTTCCTAAG TCTGGTGCGA TCGCAGTTTC AGTTTTGCGG 720  
 ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA 780  
 45 GGTGTCCACC GTCCGTTTCG CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGA 840  
 CGCCTGGTGG ACCCCTTTGA AGGCCAAGAG ACCATTTGGG GTTGCGAGAT CGTGGGTTCG 900  
 50 AGTCCCACCT CGTGCCCAGT TGCGAGATCG TGGGTTTCGAG TCCCACCTCG TGTTTTGTTG 960  
 CGAGATCGTG GGTTTCGAGT CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC 1020  
 ACCTCGTGTT TTGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT 1080  
 55 CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT 1140  
 CTGATTCTTC TGGTTTCTCT TTTTGTCTTA GTCTCGTGTC CGCTCTTGTT GTGACTACTG 1200  
 60 TTTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTGCG 1260  
 TTGGTAATTT TGTTTGTTTA CGTTTGTTTT TGTGAGTCGT CTATGTTGTC TGTTACTATC 1320  
 TTGTTTTTGT TTGTGGTTTA CGGTTTCTGT GTGTGTCTTG TGTGTCTCTT TGTGTTTCTA 1380  
 65 CTTGGACTGA TGAAGTACGA CTGTTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAAATC 1440

	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTT	ACTCGAGCTC	1500
5	AAGCTTCGAA	TTCTGCAGTC	GACGGTACCG	CGGGCCCGGG	ATCCACCGGT	CGCCACCATG	1560
	GGTAAAGGAG	AAGAACTTTT	CACAGGAGTT	GTCCCAATTC	TTGTTGAATT	AGATGGTGAT	1620
	GTTAATGGGC	ACAAATTTTC	TGTCAGTGGA	GAGGGTGAAG	GTGATGCAAC	ATACGGAAAA	1680
10	CTTACCCTTA	AATTTATTTG	CACTACTGGA	AAACTACCTG	TTCCATGGCC	AACACTTGTC	1740
	ACTACTTTCA	CTTATGGTGT	TCAATGCTTT	TCAAGATACC	CAGATCATAT	GAAACGGCAT	1800
15	GACTTTTTCA	AGAGTGCCAT	GCCCGAAGGT	TATGTACAGG	AAAGAACTAT	ATTTTTCAAA	1860
	GATGACGGGA	ACTACAAGAC	ACGTGCTGAA	GTCAAGTTTG	AAGGTGATAC	CCTTGTTAAT	1920
	AGAATCGAGT	TAAAAGGTAT	TGATTTTAAA	GAAGATGGAA	ACATTCTTGG	ACACAAATTG	1980
20	GAATACAAC	ATAACTCACA	CAATGTATAC	ATCATGGCAG	ACAAACAAAA	GAATGGAACC	2040
	AAAGTTAACT	TCAAAATTAG	ACACAACATT	GAAGATGGAA	GCGTTCAACT	AGCAGACCAT	2100
25	TATCAACAAA	ATACTCCAAT	TGGCGATGGC	CCTGTCCTTT	TACCAGACAA	CCATTACCTG	2160
	TCCACACAAT	CTGCCCTTTC	GAAAGATCCC	AACGAAAAGA	GAGACCACAT	GGTCCTTCTT	2220
	GAGTTTGTA	CAGCTGCTGG	GATTACACAT	GGCATGGATG	AACTATACAA	GTCCGGATCT	2280
30	AGATAACTGT	ATCGATGGAT	CCGAAGGCGG	GGACAGCAGT	GCAGTGGTGG	ACAGAAAGCA	2340
	AGTGATCTAG	GCCAGCAGCC	TCCCTAAAGG	GACTTCAGCC	CACAAAGCCA	AACTTGTTGGC	2400
35	TTTAATACAA	GCTCTGTAAA	TGGTAAAAAA	AAAAAAGTCT	ACACGGACAG	CAGGTATGCT	2460
	CTTGCCACTG	TACAGAGCAA	TATACAGACA	AAGAGAACTG	TTGACATCTG	CAGAGAAAGA	2520
	CCTAAGATGC	TGTGGCTAAA	AGAAATCAGA	TGGCAAATCT	AACCGCCCAG	GCATCCTAAA	2580
40	GAGCAATGAT	CCTGACAGTC	TGAAGACTAT	CAAGTTATAG	ACAAATTAAG	ACTGGTAAAA	2640
	AAAACCCTGT	ATAAAATAGT	AAAAACTGAA	AAAAGAAAAC	TAGTCCTCTC	ATGAGAAGAC	2700
	AGACCTGACA	TCTACTGAAA	AATAGACTTT	ACTGGAAAAA	ATATGTGTAT	GAATACCTTC	2760
45	TAGTTTTTGT	GAACGTTCTC	AAGATGGATA	AAAGCTTTTC	CTTGTAAC	GAGACTGATC	2820
	AGATAGTCAT	CAAGAAGATT	GTTAAAGAAA	ATTTTCCAAG	GTTCGGAGTG	CCAAAAGCAA	2880
50	TAGTGTCAGA	TAATGGTCCT	GCCTTTGTTG	CCCAGGTAAG	TCAGGGTGTG	GCCAAGTATT	2940
	TAGAGGTCAA	ATGAAAATTC	CATTGTGTGT	ACAGACCTCA	GAGCTCAGGA	AAGATAAAAA	3000
	AGAATAAATA	AAACTCTAAA	CAGACCTTGA	CAAAATTAAT	CCTAGAGACT	GGCACAGACT	3060
55	TACTTGGTAC	TCCTTCCCCT	TGCCCTATTT	AGAACTGAGA	ATACTCCCTC	TTGATTCCGT	3120
	TTTACTCTTT	TTAAGATCCT	TTATGGGGCT	CCTATGCCAT	CACTGTCTTA	AATGATGTGT	3180
60	TTAAACCTAT	GTTGTTATAA	TAATGATCTA	TATGTTAAGT	TAAAAGGCTT	GCAGGTGGTG	3240
	CAGAAAGAAG	TCTGGTCACA	ACTGGCTACA	GTGAACAAGC	TGGGTACCCC	AAGGACATCT	3300
	TACCAGTTCC	AGCCAGAGAT	CTGATCTACG	ATCCCCGGGT	CGACCCGGGT	CGACCCTGTG	3360
65	GAATGTGTGT	CAGTTAGGGT	GTGGAAAGTC	CCCAGGCTCC	CCAGCAGGCA	GAAGTATGCA	3420

	AAGCATGCAT	CTCAATTAGT	CAGCAACCAG	GTGTGGAAAG	TCCCCAGGCT	CCCCAGCAGG	3480
	CAGAAGTATG	CAAAGCATGC	ATCTCAATTA	GTCAGCAACC	ATAGTCCCGC	CCCTAACTCC	3540
5	GCCCATCCCC	CCCCTAACTC	CGCCCAGTTC	CGCCCATTCT	CCGCCCCATG	GCTGACTAAT	3600
	TTTTTTTTATT	TATGCAGAGG	CCGAGGCCGC	CTCGGCCTCT	GAGCTATTCC	AGAAGTAGTG	3660
	AGGAGGCTTT	TTTGGAGGCC	TAGGCTTTTG	CAAAAAGCTT	CACGCTGCCG	CAAGCACTCA	3720
10	GGGCGCAAGG	GCTGCTAAAG	GAAGCGGAAC	ACGTAGAAAAG	CCAGTCCGCA	GAAACGGTGC	3780
	TGACCCCGGA	TGAATGTCAG	CTACTGGGCT	ATCTGGACAA	GGGAAAACGC	AAGCGCAAAG	3840
15	AGAAAGCAGG	TAGCTTGACG	TGGGCTTACA	TGGCGATAGC	TAGACTGGGC	GGTTTTATGG	3900
	ACAGCAAGCG	AACCGGAATT	GCCAGCTGGG	GCGCCCTCTG	GTAAGGTTGG	GAAGCCCTGC	3960
	AAAGTAAACT	GGATGGCTTT	CTTGCCGCCA	AGGATCTGAT	GGCGCAGGGG	ATCAAGATCT	4020
20	GATCAAGAGA	CAGGATGAGG	ATCGTTTCGC	ATGATTGAAC	AAGATGGATT	GCACGCAGGT	4080
	TCTCCGGCCG	CTTGGGTGGA	GAGGCTATTC	GGCTATGACT	GGGCACAACA	GACAATCGGC	4140
25	TGCTCTGATG	CCGCCGTGTT	CCGGCTGTCA	GCGCAGGGGC	GCCCGGTTCT	TTTTGTCAAG	4200
	ACCGACCTGT	CCGGTGCCCT	GAATGAACTG	CAGGACGAGG	CAGCGCGGCT	ATCGTGGCTG	4260
	GCCACGACGG	GCGTTCCTTG	CGCAGCTGTG	CTCGACGTTG	TCACTGAAGC	GGGAAGGGAC	4320
30	TGGCTGCTAT	TGGGCGAAGT	GCCGGGGCAG	GATCTCCTGT	CATCTCACCT	TGCTCCTGCC	4380
	GAGAAAGTAT	CCATCATGGC	TGATGCAATG	CGGCGGCTGC	ATACGCTTGA	TCCGGCTACC	4440
35	TGCCCCATTCG	ACCACCAAGC	GAAACATCGC	ATCGAGCGAG	CACGTAICTG	GATGGAAGCC	4500
	GGTCTTGTCG	ATCAGGATGA	TCTGGACGAA	GAGCATCAGG	GGCTCGCGCC	AGCCGAACTG	4560
	TTCGCCAGGC	TCAAGGCGCG	CATGCCCGAC	GGCGAGGATC	TCGTCTGTGAC	CCATGGCGAT	4620
40	GCCTGCTTGC	CGAATATCAT	GGTGGAAAAT	GGCCGCTTTT	CTGGATTTCAT	CGACTGTGGC	4680
	CGGCTGGGTG	TGGCGGACCG	CTATCAGGAC	ATAGCGTTGG	CTACCCGTGA	TATTGCTGAA	4740
	GAGCTTGCGG	GCGAATGGGC	TGACCGCTTC	CTCGTGCTTT	ACGGTATCGC	CGCTCCCGAT	4800
45	TCGCAGCGCA	TCGCCTTCTA	TCGCCTTCTT	GACGAGTTCT	TCTGAGCGGG	ACTCTGGGGT	4860
	TCGAAATGAC	CGACCAAGCG	ACGCCCAACC	TGCCATCACG	AGATTTTCGAT	TCCACCGCCG	4920
50	CCTTCTATGA	AAGGTTGGGC	TTCGGAATCG	TTTTCCGGGA	CGGAATTTCG	AATCTGCTGC	4980
	TTGCAAACAA	AAAAACCACC	GCTACCAGCG	GTGGTTTGTG	TGCCGGATCA	AGAGCTACCA	5040
	ACTCTTTTTT	CGAAGGTAAC	TGGCTTCAGC	AGAGCGCAGA	TACCAAATAC	TGTCCTTCTA	5100
55	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	5160
	CTGCTAATCC	TGTTACCAGT	GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	5220
60	GACTCAAGAC	GATAGTTACC	GGATAAGGCG	CAGCGGTCGG	GCTGAACGGG	GGGTTTCGTGC	5280
	ACACAGCCCA	GCTTGAGCGG	AACGACCTAC	ACCGAACTGA	GATACCTACA	GCGTGAGCAT	5340
	TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA	AAGGCGGACA	GGTATCCGGT	AAGCGGCAGG	5400
65	GTCGGAACAG	GAGAGCGCAC	GAGGGAGCTT	CCAGGGGGAA	ACGCCTGGTA	TCTTTATAGT	5460



5 CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG 5520  
CGGAGCCTAT GGAAAAACGC CAGCAACGCC GAGATGCGCC GCCTCGAGTA CACCTGCGTC 5580  
ATGCTGAGAC CCTCAAGCCT CACTAAAAGG GTCCCTGCCT AGTTCTGTTT ACTAATCTGC 5640  
CTTATTCTGT TTTTGTTCCT ATGTTAAAGA TAGAGTAAAT GCAGTATTCT CCACATAGAG 5700  
10 ATATAGACTT CTGAAATTCT AAGATTAGAA TTATTTACAA GAAGAAGTGG GGAA 5754

(2) INFORMATION FOR SEQ ID NO:19:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5754 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGAAGAATAA AAAATTACTG GCCTCTTGTT AGAACATGAA CTTTCACCTC GGAGCCCACC 60  
CCCTCCCATC TGGAACACAT ACTTGAGAAA AACATTTTCT GGAACAACCA CAGAATGTTT 120  
30 CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG 180  
TTGTAAACT TCCCCTATTG CCTCCCCATT CCCCCTCCCA GTTTGTGGTT TTTTCCTTTA 240  
35 AAAGCTTGTT AAAAATTTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG 300  
AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTTTCGAC CCCAGAGCTC 360  
TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG 420  
40 GTCTCAGTGT CTTCTTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT 480  
TCCCTCGAGG GTCTTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG 540  
45 CATTGGCCGG GAATTCGAAA ATCTTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA 600  
CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTTGT TCTGTTTGG TCTGATGTCT 660  
GTGTTCTGAT GTCTGTGTTT TGTCTCTAAG TCTGGTGCGA TCGCAGTTT AGTTTTGCGG 720  
50 ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA 780  
GGTGTCCACC GTCCGTTTCG CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGA 840  
55 CGCCTGGTGG ACCCCTTTGA AGGCCAAGAG ACCATTTGGG GTTGCAGAT CGTGGGTTTCG 900  
AGTCCCACCT CGTGCCAGT TGCAGATCG TGGGTTTCAG TCCCACCTCG TGTTTTGTTG 960  
CGAGATCGTG GGTTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC 1020  
60 ACCTCGTGTT TTGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT 1080  
CGTGGGTTTC AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT 1140  
65 CTGATTCTTC TGGTTTCTCT TTTTGTCTTA GTCTCGTGTC CGCTCTTGTT GTGACTACTG 1200

	TTTTTCTAAA	AATGGGACAA	TCTGTGTCCA	CTCCCCTTTC	TCTGACTCTG	GTTCTGTGCG	1260
	TTGGTAATTT	TGTTTGTTTA	CGTTTGTTTT	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	1320
5	TTGTTTTTGT	TTGTGGTTTA	CGGTTTCTGT	GTGTGTCTTG	TGTGTCTCTT	TGTGTTTCTG	1380
	CTTGGACTGA	TGACTGACGA	CTGTTTTTTAA	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	1440
10	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTT	ACTCGAGCTC	1500
	AAGCTTCGAA	TTCTGCAGTC	GACGGTACCG	CGGGCCCCGG	ATCCACCGGT	CGCCACCATG	1560
	GGTAAAGGAG	AAGAACTTTT	CACTGGAGTT	GTCCCAATTC	TTGTTGAATT	AGATGGTGAT	1620
15	GTTAATGGGC	ACAAATTTTC	TGTCAGTGGA	GAGGGTGAAG	GTGATGCAAC	ATACGGAAAA	1680
	CTTACCCTTA	AATTTATTTG	CACTACTGGA	AACTACCTG	TTCCATGGCC	AACACTTGTC	1740
20	ACTACTTTCT	CTTATGGTGT	TCAATGCTTT	TCAAGATACC	CAGATCATAT	GAAACGGCAT	1800
	GACTTTTTTCA	AGAGTGCCAT	GCCCGAAGGT	TATGTACAGG	AAAGAACTAT	ATTTTTCTAA	1860
	GATGACGGGA	ACTACAAGAC	ACGTGCTGAA	GTCAAGTTTG	AAGGTGATAC	CCTTGTTAAT	1920
25	AGAATCGAGT	TAAAAGGTAT	TGATTTTAAA	GAAGATGGAA	ACATTCTTGG	ACACAAATTG	1980
	GAATACAAC	ATAACTCACA	CAATGTATAC	ATCATGGCAG	ACAAACAAAA	GAATGGAACC	2040
30	AAAGTTAACT	TCAAAATTAG	ACACAACATT	GAAGATGGAA	GCGTTCAACT	AGCAGACCAT	2100
	TATCAACAAA	ATACTCCAAT	TGGCGATGGC	CCTGTCTCTT	TACCAGACAA	CCATTACCTG	2160
	TCCACACAAT	CTGCCCTTTC	GAAAGATCCC	AACGAAAAGA	GAGACCACAT	GGTCCTTCTT	2220
35	GAGTTTGTA	CAGCTGCTGG	GATTACACAT	GGCATGGATG	AACTATACAA	GTCCGGATCT	2280
	AGATAACTGT	ATCGATGGAT	CCGAAGGCGG	GGACAGCAGT	GCAGTGGTGG	ACAGAAAGCA	2340
	AGTGATCTAG	GCCAGCAGCC	TCCCTAAAGG	GACTTCAGCC	CACAAAGCCA	AACTTGTTGG	2400
40	TTTAATACAA	GCTCTGTAAA	TGGTAAAAAA	AAAAAAGTCT	ACACGGACAG	CAGGTATGCT	2460
	CTTGCCACTG	TACAGAGCAA	TATACAGACA	AAGAGAACTG	TTGACATCTG	CAGAGAAAGA	2520
45	CCTAAGATGC	TGTGGCTAAA	AGAAATCAGA	TGGCAAATCT	AACCGCCCAG	GCATCCTAAA	2580
	GAGCAATGAT	CCTGACAGTC	TGAAGACTAT	CAAGTTATAG	ACAAATTAAG	ACTGGTAAAA	2640
	AAAACCCTGT	ATAAAATAGT	AAAAACTGAA	AAAAGAAAAC	TAGTCCTCTC	ATGAGAAGAC	2700
50	AGACCTGACA	TCTACTGAAA	AATAGACTTT	ACTGGAAAAA	ATATGTGTAT	GAATACCTTC	2760
	TAGTTTTTGT	GAACGTTCTC	AAGATGGATA	AAAGCTTTTC	CTTGTAATAA	GAGACTGATC	2820
55	AGATAGTCAT	CAAGAAGATT	GTAAAGAAA	ATTTTCCAAG	GTTTCGGAGT	CCAAAGCAA	2880
	TAGTGTGAGA	TAATGGTCCT	GCCTTTGTTG	CCCAGGTAAG	TCAGGGTGTG	GCCAAGTATT	2940
	TAGAGGTCAA	ATGAAAATTC	CATTGTGTGT	ACAGACCTCA	GAGCTCAGGA	AAGATAAAAA	3000
60	AGAATAAATA	AAACTCTAAA	CAGACCTTGA	CAAAATTAAT	CCTAGAGACT	GGCACAGACT	3060
	TACTTGGTAC	TCCTTCCCCT	TGCCCTATTT	AGAACTGAGA	ATACTCCCTC	TTGATTCGGT	3120
65	TTTACTCTTT	TTAAGATCCT	TTATGGGGCT	CCTATGCCAT	CACTGTCTTA	AATGATGTGT	3180
	TTAAACCTAT	GTTGTTATAA	TAATGATCTA	TATGTTAAGT	TAAAAGGCTT	GCAGGTGGTG	3240

	CAGAAAGAAG	TCTGGTCACA	ACTGGCTACA	GTGAACAAGC	TGGGTACCCC	AAGGACATCT	3300
5	TACCAGTTCC	AGCCAGAGAT	CTGATCTACG	ATCCCCGGGT	CGACCCGGGT	CGACCCTGTG	3360
	GAATGTGTGT	CAGTTAGGGT	GTGGAAAGTC	CCCAGGCTCC	CCAGCAGGCA	GAAGTATGCA	3420
	AAGCATGCAT	CTCAATTAGT	CAGCAACCAG	GTGTGGAAAG	TCCCCAGGCT	CCCCAGCAGG	3480
10	CAGAAGTATG	CAAAGCATGC	ATCTCAATTA	GTCAGCAACC	ATAGTCCCGC	CCCTAACTCC	3540
	GCCCATCCCC	CCCCTAACTC	CGCCCAGTTC	CGCCCATTCT	CCGCCCCATG	GCTGACTAAT	3600
15	TTTTTTTTATT	TATGCAGAGG	CCGAGGCCGC	CTCGGCCTCT	GAGCTATTCC	AGAAGTAGTG	3660
	AGGAGGCTTT	TTTGGAGGCC	TAGGCTTTTG	CAAAAAGCTT	CACGCTGCCG	CAAGCACTCA	3720
	GGGCGCAAGG	GCTGCTAAAG	GAAGCGGAAC	ACGTAGAAAG	CCAGTCCGCA	GAAACGGTGC	3780
20	TGACCCCGGA	TGAATGTCAG	CTACTGGGCT	ATCTGGACAA	GGGAAAACGC	AAGCGCAAAG	3840
	AGAAAGCAGG	TAGCTTGAG	TGGGCTTACA	TGGCGATAGC	TAGACTGGGC	GGTTTTATGG	3900
25	ACAGCAAGCG	AACCGGAATT	GCCAGCTGGG	GCGCCCTCTG	GTAAGGTTGG	GAAGCCCTGC	3960
	AAAGTAAACT	GGATGGCTTT	CTTGCCGCCA	AGGATCTGAT	GGCGCAGGGG	ATCAAGATCT	4020
	GATCAAGAGA	CAGGATGAGG	ATCGTTTCGC	ATGATTGAAC	AAGATGGATT	GCACGCAGGT	4080
30	TCTCCGGCCG	CTTGGGTGGA	GAGGCTATTC	GGCTATGACT	GGGCACAACA	GACAATCGGC	4140
	TGCTCTGATG	CCGCCGTGTT	CCGGCTGTCA	GCGCAGGGGC	GCCCGGTTCT	TTTTGTCAAG	4200
35	ACCGACCTGT	CCGGTGCCCT	GAATGAACTG	CAGGACGAGG	CAGCGCGGCT	ATCGTGGCTG	4260
	GCCACGACGG	GCGTTCCTTG	CGCAGCTGTG	CTCGACGTTG	TCACTGAAGC	GGGAAGGGAC	4320
	TGGCTGCTAT	TGGGCGAAGT	GCCGGGGCAG	GATCTCCTGT	CATCTCACCT	TGCTCCTGCC	4380
40	GAGAAAGTAT	CCATCATGGC	TGATGCAATG	CGGCGGCTGC	ATACGCTTGA	TCCGGCTACC	4440
	TGCCCCATTCG	ACCACCAAGC	GAAACATCGC	ATCGAGCGAG	CACGTACTCG	GATGGAAGCC	4500
	GGTCTTGTCG	ATCAGGATGA	TCTGGACGAA	GAGCATCAGG	GGCTCGCGCC	AGCCGAACTG	4560
45	TTCGCCAGGC	TCAAGGCGCG	CATGCCCCGAC	GGCGAGGATC	TCGTCGTGAC	CCATGGCGAT	4620
	GCCTGCTTGC	CGAATATCAT	GGTGGAAAAT	GGCCGCTTTT	CTGGATTTCAT	CGACTGTGGC	4680
50	CGGCTGGGTG	TGGCGGACCG	CTATCAGGAC	ATAGCGTTGG	CTACCCGTGA	TATTGCTGAA	4740
	GAGCTTGGCG	GCGAATGGGC	TGACCGCTTC	CTCGTGCTTT	ACGGTATCGC	CGCTCCCGAT	4800
	TCGCAGCGCA	TCGCCTTCTA	TCGCCTTCTT	GACGAGTTCT	TCTGAGCGGG	ACTCTGGGGT	4860
55	TCGAAATGAC	CGACCAAGCG	ACGCCCAACC	TGCCATCACG	AGATTTTCGAT	TCCACCGCCG	4920
	CCTTCTATGA	AAGGTTGGGC	TTCGGAATCG	TTTTCCGGGA	CGGAATTTCG	AATCTGCTGC	4980
60	TTGCAAACAA	AAAAACCACC	GCTACCAGCG	GTGGTTTGTG	TGCCGGATCA	AGAGCTACCA	5040
	ACTCTTTTTC	CGAAGGTAAC	TGGCTTCAGC	AGAGCGCAGA	TACCAAATAC	TGTCTTCTTA	5100
	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	5160
65	CTGCTAATCC	TGTTACCAGT	GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	5220

GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC 5280  
 ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCAT 5340  
 5 TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG 5400  
 GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT 5460  
 CCTGTGCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG 5520  
 10 CGGAGCCTAT GGAAAAACGC CAGCAACGCC GAGATGCGCC GCCTCGAGTA CACCTGCGTC 5580  
 ATGCTGAGAC CCTCAAGCCT CACTAAAAGG GTCCCTGCCT AGTTCTGTTT ACTAATCTGC 5640  
 15 CTTATTCTGT TTTTGTTCCT ATGTTAAAGA TAGAGTAAAT GCAGTATTCT CCACATAGAG 5700  
 ATATAGACTT CTGAAATTCT AAGATTAGAA TTATTTACAA GAAGAAGTGG GGAA 5754

(2) INFORMATION FOR SEQ ID NO:20:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4958 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 AGGCGGGGAC AGCAGTGCAG TGGTGGACAG AAAGCAAGTG ATCTAGGCCA GCAGCCTCCC 60  
 TAAAGGGACT TCAGCCCACA AAGCCAACT TGTGGCTTTA ATACAAGCTC TGTAATGGT 120  
 AAAAAAAAAA AAGTCTACAC GGACAGCAGG TATGCTCTTG CCACTGTACA GAGCAATATA 180  
 40 CAGACAAAGA GAACTGTTGA CATCTGCAGA GAAAGACCTA AGATGCTGTG GCTAAAAGAA 240  
 ATCAGATGGC AAATCTAACC GCCCAGGCAT CCTAAAGAGC AATGATCCTG ACAGTCTGAA 300  
 GACTATCAAG TTATAGACAA ATTAAGACTG GTAAAAAAA CCCTGTATAA AATAGTAAAA 360  
 45 ACTGAAAAAA GAAACTAGT CCTCTCATGA GAAGACAGAC CTGACATCTA CTGAAAAATA 420  
 GACTTTACTG GAAAAAATAT GTGTATGAAT ACCTTCTAGT TTTTGTGAAC GTTCTCAAGA 480  
 50 TGGATAAAAG CTTTTCCTTG TAAAACGAGA CTGATCAGAT AGTCATCAAG AAGATTGTTA 540  
 AAGAAAATTT TCCAAGGTTT GGAGTGCCAA AAGCAATAGT GTCAGATAAT GGTCTGCCT 600  
 TTGTTGCCCA GGTAAGTCAG GGTGTGGCCA AGTATTTAGA GGTCAAATGA AAATTCCATT 660  
 55 GTGTGTACAG ACCTCAGAGC TCAGGAAAGA TAAAAAGAA TAAATAAAAC TCTAAACAGA 720  
 CCTTGACAAA ATTAATCCTA GAGACTGGCA CAGACTTACT TGGTACTCCT TCCCCCTGCC 780  
 60 CTATTTAGAA CTGAGAATAC TCCCTCTTGA TTCGGTTTTA CTCTTTTTTA GATCCTTTAT 840  
 GGGGCTCCTA TGCCATCACT GTCTTAAATG ATGTGTTTAA ACCTATGTTG TTATAATAAT 900  
 GATCTATATG TTAAGTTAAA AGGCTTGCAG GTGGTGCAGA AAGAAGTCTG GTCACAACTG 960  
 65 GCTACAGTGA ACAAGCTGGG TACCCCAAGG ACATCTTACC AGTTCCAGCC AGAGATCTGA 1020

	TCTACGATCC	CCGGGTCGAC	CCGGGTCGAC	CCTGTGGAAT	GTGTGTCAGT	TAGGGTGTGG	1080
5	AAAGTCCCCA	GGCTCCCCAG	CAGGCAGAAG	TATGCAAAGC	ATGCATCTCA	ATTAGTCAGC	1140
	AACCAGGTGT	GGAAAGTCCC	CAGGCTCCCC	AGCAGGCAGA	AGTATGCAAA	GCATGCATCT	1200
	CAATTAGTCA	GCAACCATAG	TCCCGCCCCCT	AACTCCGCCC	ATCCCGCCCC	TAACTCCGCC	1260
10	CAGTTCGCC	CATTCTCCGC	CCCATGGCTG	ACTAATTTTT	TTTATTTATG	CAGAGGCCGA	1320
	GGCCGCCTCG	GCCTCTGAGC	TATTCCAGAA	GTAGTGAGGA	GGCTTTTTTG	GAGGCCTAGG	1380
15	CTTTTGCAAA	AAGCTTCACG	CTGCCGCAAG	CACTCAGGGC	GCAAGGGCTG	CTAAAGGAAG	1440
	CGGAACACGT	AGAAAGCCAG	TCCGCAGAAA	CGGTGCTGAC	CCCGGATGAA	TGTCAGCTAC	1500
	TGGGCTATCT	GGACAAGGGA	AAACGCAAGC	GCAAAGAGAA	AGCAGGTAGC	TTGCAGTGGG	1560
20	CTTACATGGC	GATAGCTAGA	CTGGGCGGTT	TTATGGACAG	CAAGCGAACC	GGAATTGCCA	1620
	GCTGGGGCGC	CCTCTGGTAA	GGTTGGGAAG	CCCTGCAAAG	TAAACTGGAT	GGCTTTCTTG	1680
25	CCGCCAAGGA	TCTGATGGCG	CAGGGGATCA	AGATCTGATC	AAGAGACAGG	ATGAGGATCG	1740
	TTTCGCATGA	TTGAACAAGA	TGGATTGCAC	GCAGGTTCTC	CGGCCGCTTG	GGTGGAGAGG	1800
	CTATTCGGCT	ATGACTGGGC	ACAACAGACA	ATCGGCTGCT	CTGATGCCGC	CGTGTTCGGG	1860
30	CTGTCAGCGC	AGGGGCGCCC	GGTTCTTTTT	GTCAAGACCG	ACCTGTCCGG	TGCCCTGAAT	1920
	GAACTGCAGG	ACGAGGCAGC	GCGGCTATCG	TGGCTGGCCA	CGACGGGCGT	TCCTTGCGCA	1980
35	GCTGTGCTCG	ACGTTGTCAC	TGAAGCGGGA	AGGGACTGGC	TGCTATTGGG	CGAAGTGCCG	2040
	GGGCAGGATC	TCCTGTTCATC	TCACCTTGCT	CCTGCCGAGA	AAGTATCCAT	CATGGCTGAT	2100
	GCAATGCGGC	GGCTGCATAC	GCTTGATCCG	GCTACCTGCC	CATTCGACCA	CCAAGCGAAA	2160
40	CATCGCATCG	AGCGAGCACG	TACTCGGATG	GAAGCCGGTC	TTGTGATCA	GGATGATCTG	2220
	GACGAAGAGC	ATCAGGGGCT	CGCGCCAGCC	GAAGTGTTCG	CCAGGCTCAA	GGCGCGCATG	2280
	CCCGACGGCG	AGGATCTCGT	CGTGACCCAT	GGCGATGCCT	GCTTGCCGAA	TATCATGGTG	2340
45	GAAAAATGGCC	GCTTTTCTGG	ATTTCATCGAC	TGTGGCCGGC	TGGGTGTGGC	GGACCGCTAT	2400
	CAGGACATAG	CGTTGGCTAC	CCGTGATATT	GCTGAAGAGC	TTGGCGGCGA	ATGGGCTGAC	2460
50	CGCTTCCTCG	TGCTTTACGG	TATCGCCGCT	CCCGATTTCG	AGCGCATCGC	CTTCTATCGC	2520
	CTTCTTGACG	AGTTCTTCTG	AGCGGGACTC	TGGGGTTCGA	AATGACCGAC	CAAGCGACGC	2580
	CCAACCTGCC	ATCACGAGAT	TTCGATTCCA	CCGCCGCCTT	CTATGAAAGG	TTGGGCTTCG	2640
55	GAATCGTTTT	CCGGGACGGA	ATTTCGTAATC	TGCTGCTTGC	AAACAAAAAA	ACCACCGCTA	2700
	CCAGCGGTGG	TTTGTGTTGCC	GGATCAAGAG	CTACCAACTC	TTTTTCCGAA	GGTAACTGGC	2760
60	TTCAGCAGAG	CGCAGATACC	AAATACTGTC	CTTCTAGTGT	AGCCGTAGTT	AGGCCACCAC	2820
	TTCAAGAACT	CTGTAGCACC	GCCTACATAC	CTCGCTCTGC	TAATCCTGTT	ACCAGTGGCT	2880
	GCTGCCAGTG	GCGATAAGTC	GTGTCTTACC	GGGTTGGACT	CAAGACGATA	GTTACCGGAT	2940
65	AAGGCGCAGC	GGTCGGGCTG	AACGGGGGGT	TCGTGCACAC	AGCCCAGCTT	GGAGCGAACC	3000

	ACCTACACCG	AACTGAGATA	CCTACAGCGT	GAGCATTGAG	AAAGCGCCAC	GCTTCCCCGAA	3060
	GGGAGAAAGG	CGGACAGGTA	TCCGGTAAGC	GGCAGGGTCG	GAACAGGAGA	GCGCACGAGG	3120
5	GAGCTTCCAG	GGGGAAACGC	CTGGTATCTT	TATAGTCCTG	TCGGGTTTCG	CCACCTCTGA	3180
	CTTGAGCGTC	GATTTTTGTG	ATGCTCGTCA	GGGGGGCGGA	GCCTATGGAA	AAACGCCAGC	3240
	AACGCCGAGA	TGCGCCGCCT	CGAGTACACC	TGCGTCATGC	TGAGACCCTC	AAGCCTCACT	3300
10	AAAAGGGTCC	CTGCCTAGTT	CTGTTTACTA	ATCTGCCTTA	TTCTGTTTTT	GTTCCCATGT	3360
	TAAAGATAGA	GTAAATGCAG	TATTCTCCAC	ATAGAGATAT	AGACTTCTGA	AATTCTAAGA	3420
15	TTAGAATTAT	TTACAAGAAG	AAGTGGGGAA	TGAAGAATAA	AAAATTACTG	GCCTCTTG TG	3480
	AGAACATGAA	CTTTCACCTC	GGAGCCCACC	CCCTCCCATC	TGGAAAACAT	ACTTGAGAAA	3540
	AACATTTTCT	GGAACAACCA	CAGAATGTTT	CAACAGGCCA	GATGTATTGC	CAAACACAGG	3600
20	ATATGACTCT	TTGGTTGAGT	AAATTTGTGG	TTGTTAAACT	TCCCCTATTC	CCTCCCCATT	3660
	CCCCCTCCCA	GTTTGTGGTT	TTTTCCTTTA	AAAGCTTG TG	AAAAATTTGA	GTCGTCGT CG	3720
25	AGACTCCTCT	ACCCTGTGCA	AAGGTGTATG	AGTTTCGACC	CCAGAGCTCT	GTGTGCTTTC	3780
	TGTTGCTGCT	TTATTTCGAC	CCCAGAGCTC	TGGTCTGTGT	GCTTTCATGT	CGCTGCTTTA	3840
	TTAAATCTTA	CCTTCTACAT	TTTATGTATG	GTCTCAGTGT	CTTCTGGGT	ACGCGGCTGT	3900
30	CCCGGGACTT	GAGTGTCTGA	GTGAGGGTCT	TCCCTCGAGG	GTCTTTCATT	TGGTACATGG	3960
	GCCGGGAATT	CGAGAATCTT	TCATTTGGTG	CATTGGCCGG	GAATTCGAAA	ATCTTTCATT	4020
35	TGGTGCAATTG	GCCGGGAAAC	AGCGCGACCA	CCCAGAGGTC	CTAGACCCAC	TTAGAGGTAA	4080
	GATTCTTTGT	TCTGTTTTTG	TCTGATGTCT	GTGTTCTGAT	GTCTGTGTTT	TGTTTCTAAG	4140
	TCTGGTGCGA	TCGCAGTTTC	AGTTTTGCGG	ACGCTCAGTG	AGACCGCGCT	CCGAGAGGGA	4200
40	GTGCGGGGTG	GATAAGGATA	GACGTGTCCA	GGTGTCCACC	GTCCGTTTCG	CCTGGGAGAC	4260
	GTCCCAGGAG	GAACAGGGGA	GGATCAGGGA	CGCCTGGTGG	ACCCCTTTGA	AGGCCAAGAG	4320
	ACCATTTGGG	GTTGCGAGAT	CGTGGGTTCG	AGTCCCACCT	CGTGCCCAGT	TGCGAGATCG	4380
45	TGGGTTCGAG	TCCCACCTCG	TGTTTTGT TG	CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	4440
	TCTGGTCACG	GGATCGTGGG	TTGAGTCCC	ACCTCGTGTT	TTGTTGCGAG	ATCGTGGGTT	4500
50	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	CGTGGGTTCG	AGTCCCACCT	CGTGCAGAGG	4560
	GTCTCAATTG	GCCGGCCTTA	GAGAGGCCAT	CTGATTCTTC	TGGTTTCTCT	TTTTGTCTTA	4620
55	GTCTCGTGTC	CGCTCTTGTT	GTGACTACTG	TTTTTCTAAA	AATGGGACAA	TCTGTGTCCA	4680
	CTCCCCTTTC	TCTGACTCTG	GTTCTGTGCG	TTGGTAATTT	TGTTTGTTTA	CGTTTGTTTT	4740
	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	TTGTTTTTGT	TTGTGGTTTA	CGGTTTCTGT	4800
60	GTGTGTCTTG	TGTGTCTCTT	TGTGTTTACA	CTTGGACTGA	TGACTGACGA	CTGTTTTTAA	4860
	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	4920
65	TCAGATCAAC	AGCTGCCCTT	ACGTATCGAT	GGATCCGA			4958

(2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7080 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

15	GAATACAAGC TTGCATGCCT GCAGGTCGAC TCTAGAGGAT CTTGAAGAAT AAAAAATTAC	60
	TGGCCTCTTG TGAGAACATG AACTTTCACC TCGGAGCCCA CCCCCTCCCA TCTGAAAAAC	120
	ATACTTGAGA AAAACATTTT CTGGAACAAC CACAGAATGT TTCAACAGGC CAGATGTATT	180
20	GCCAAACACA GGATATGACT CTTTGGTTGA GTAAATTTGT GGTGTGTTAA CTTCCCCTAT	240
	TCCCTCCCA TTCCCCTCC CAGTTTGTGG TTTTTCCTT TAAAAGCTTG TGAAAAATTT	300
25	GAGTCGTCGT CGAGACTCCT CTACCCTGTG CAAAGGTGTA TGAGTTTCGA CCCAGAGCT	360
	CTGTGTGCTT TCTGTTGCTG CTTTATTTCTG ACCCCAGAGC TCTGGTCTGT GTGCTTTCAT	420
	GTCGCTGCTT TATTAAATCT TACCTTCTAC ATTTTATGTA TGGTCTCAGT GTCTTCTTGG	480
30	GTACGCGGCT GTCCCGGGAC TTGAGTGTCT GAGTGAGGGT CTTCCCTCGA GGGTCTTTCA	540
	TTTGGTACAT GGGCCGGGAA TTCGAGAATC TTTCATTTGG TGCATTGGCC GGGAATTCGA	600
35	AAATCTTTCA TTTGGTGCAT TGGCCGGGAA ACAGCGCGAC CACCCAGAGG TCCTAGACCC	660
	ACTTAGAGGT AAGATTCTTT GTTCTGTTTT GGTCTGATGT CTGTGTTCTG ATGTCTGTGT	720
	TCTGTTTCTA AGTCTGGTGC GATCGCAGTT TCAGTTTTGC GGACGCTCAG TGAGACCGCG	780
40	CTCCGAGAGG GAGTGCGGGG TGGATAAGGA TAGACGTGTC CAGGTGTCCA CCGTCCGTTT	840
	GCCCTGGGAG ACGTCCCAGG AGGAACAGGG GAGGATCAGG GACGCCTGGT GGACCCCTTT	900
45	GAAGGCCAAG AGACCATTG GGGTTGCGAG ATCGTGGGTT CGAGTCCCAC CATCGATGGT	960
	GCAGAGGGTC TCAATTGGCC GGCCTTAGAA TTACGGATCT AGCATGATTG AACAAGATGG	1020
	ATTGCACGCA GGTCTCCGG CCGCTTGGGT GGAGAGGCTA TTCGGCTATG ACTGGGCACA	1080
50	ACAGACAATC GGCTGCTCTG ATGCCGCCGT GTTCCGGCTG TCAGCGCAGG GGCGCCCGGT	1140
	TCTTTTTGTC AAGACCGACC TGTCCGGTGC CCTGAATGAA CTGCAGGACG AGGCAGCGCG	1200
55	GCTATCGTGG CTGGCCACGA CGGGCGTTCC TTGCGCAGCT GTGCTCGACG TTGTCACTGA	1260
	AGCGGGAAGG GACTGGCTGC TATTGGGCGA AGTGCCGGGG CAGGATCTCC TGTCATCTCA	1320
	CCTTGCTCCT GCCGAGAAAG TATCCATCAT GGCTGATGCA ATGCGGCGGC TGCATACGCT	1380
60	TGATCCGGCT ACCTGCCCAT TCGACCACCA AGCGAAACAT CGCATCGAGC GAGCACGTAC	1440
	TCGGATGGAA GCCGGTCTTG TCGATCAGGA TGATCTGGAC GAAGAGCATC AGGGGCTCGC	1500
65	GCCAGCCGAA CTGTTCGCCA GGCTCAAGGC GCGCATGCCC GACGGCGAGG ATCTCGTCGT	1560

	GACCCATGGC	GATGCCTGCT	TGCCGAATAT	CATGGTGGAA	AATGGCCGCT	TTTCTGGATT	1620
	CATCGACTGT	GGCCGGCTGG	GTGTGGCGGA	CCGCTATCAG	GACATAGCGT	TGGCTACCCG	1680
5	TGATATTGCT	GAAGAGCTTG	GCGGCGAATG	GGCTGACCGC	TTCTCTGTGC	TTTACGGTAT	1740
	CGCCGCTCCC	GATTCGCAGC	GCATCGCCTT	CTATCGCCTT	CTTGACGAGT	TCTTCTGAGC	1800
10	GGGACTCTGG	GGTTCGTAAT	GACCGACCAA	GCGACGCCCA	ACCTGCCATC	ACGAGATTTT	1860
	GATTCCACCG	CCGCCTTCTA	TGAAAGGTTG	GGCTTCGGAG	TTAGCTTGTT	TCTTTACTGT	1920
	TTGTCAATTC	TATTATTTCA	ATACAGAACA	ATAGCTTCTA	TAACTGAAAT	ATATTTGCTA	1980
15	TTGTATATTA	TGATTGTCCC	TCGAACCATG	AACACTCCTC	CAGCTGAATT	TCACAATTCC	2040
	TCTGTCATCT	GCCAGGCCAT	TAAGTTATTC	ATGGAAGATC	TTGAGGAAC	ACTGCAAGTT	2100
20	CATATCATAA	ACACATTTGA	AATTGAGTAT	TGTTTTGCAT	TGTATGGAGC	TATGTTTTGC	2160
	TGTATCCTCA	GAAAAAAGT	TTGTTATAAA	GCATTCACAC	CCATAAAAAG	ATAGATTTAA	2220
	ATATTCCAGC	TATAGGAAAG	AAAGTGCGTC	TGCTCTTCAC	TCTAGTCTCA	GTTGGCTCCT	2280
25	TCACATGCAT	GCTTCTTTAT	TTCTCCTATT	TTGTCAAGAA	AATAATAGGT	CACGTCTTGT	2340
	TCTCACTTAT	GTCCTGCCTA	GCATGGCTCA	GATGCACGTT	GTAGATACAA	GAAGGATCAA	2400
30	ATGAAACAGA	CTTCTGGTCT	GTTACTACAA	CCATAGTAAT	AAGCACACTA	ACTAATAATT	2460
	GCTAATTATG	TTTTCCATCT	CTAAGGTTCC	CACATTTTTT	TGTTTTCTTA	AAGATCCCAT	2520
	TATCTGGTTG	TAACTGAAGC	TCAATGGAAC	ATGAGCAATA	TTTCCCAGTC	TTCTCTCCCA	2580
35	TCCAACAGTC	CTGATGGATT	AGCAGAACAG	GCAGAAAACA	CATTGTTACC	CAGAATTAAA	2640
	AACTAATATT	TGCTCTCCAT	TCAATCCAAA	ATGGACCTAT	TGAAACTAAA	ATCTAACCCA	2700
	ATCCCATTAA	ATGATTTCTA	TGGCGTCAAA	GGTCAAACCT	CTGAAGGGAA	CCTGTGGGTG	2760
40	GGTCACAATT	CAGGCTATAT	ATTCCCCAGG	GCTCAGCCAG	TGTCTGTACA	TACACAACGG	2820
	ATCCTGTGGA	CAGCTCACCT	AGCTGCAATG	GCTACAGGCT	CCCGGACGTC	CCTGCTCCTG	2880
45	GCTTTTGGCC	TGCTCTGCCT	GCCCTGGCTT	CAAGAGGGCA	GTGCCTTCCC	AACCATTCCC	2940
	TTATCCAGGC	TTTTTGACAA	CGCTATGCTC	CGCGCCCATC	GTCTGCACCA	GCTGGCCTTT	3000
	GACACCTACC	AGGAGTTTGA	AGAAGCCTAT	ATCCCAAAGG	AACAGAAGTA	TTCATTCCCTG	3060
50	CAGAACCCCC	AGACCTCCCT	CTGTTTCTCA	GAGTCTATT	CGACACCCCTC	CAACAGGGAG	3120
	GAAACACAAC	AGAAATCCAA	CCTAGAGCTG	CTCCGCATCT	CCCTGCTGCT	CATCCAGTCG	3180
55	TGGCTGGAGC	CCGTGCAGTT	CCTCAGGAGT	GTCTTCGCCA	ACAGCCTGGT	GTACGGCGCC	3240
	TCTGACAGCA	ACGTCTATGA	CCTCCTAAAG	GACCTAGAGG	AAGGCATCCA	AACGCTGATG	3300
	GGGAGGCTGG	AAGATGGCAG	CCCCCGGACT	GGGCAGATCT	TCAAGCAGAC	CTACAGCAAG	3360
60	TTCGACACAA	ACTCACACAA	CGATGACGCA	CTACTCAAGA	ACTACGGGCT	GCTCTACTGC	3420
	TTCAGGAAGG	ACATGGACAA	GGTCGAGACA	TTCCTGCGCA	TCGTGCAGTG	CCGCTCTGTG	3480
65	GAGGGCAGCT	GTGGCTTCTA	GCTGCCCCGG	TGGCATCCTG	TGACCCCTCC	CCAGTGCCCTC	3540
	TCCTGGCCCT	GGAAGTTGCC	ACTCCAGTGC	CCACCAGCCT	TGTCCTAATA	AAATTAAGTT	3600



	GCATCAAAAA	AAAAAAAAAAG	CTAGCGGCCG	CTAGACTTCT	GAAATTCTAA	GATTAGAATT	3660
	ATTTACAAGA	AGAAGTGGGG	AATGAAGAAT	AAAAAATTAC	TGGCCTCTTG	TGAGAACATG	3720
5	AACTTTCACC	TCGGAGCCCA	CCCCCTCCCA	TCTGGAAAAC	ATACTTGAGA	AAAACATTTT	3780
	CTGGAACAAC	CACAGAATGT	TTCAACAGGC	CAGATGTATT	GCCAAACACA	GGATATGACT	3840
10	CTTTGGTTGA	GTAAATTTGT	GGTTGTTAAA	CTTCCCCTAT	TCCCTCCCCA	TTCCCCCTCC	3900
	CAGTTTGTGG	TTTTTTCCTT	TAAAAGCTTG	TGAAAAATTT	GAGTCGTCGT	CGAGACTCCT	3960
	CTACCCTGTG	CAAAGGTGTA	TGAGTTTCGA	CCCCAGAGCT	CTGTGTGCTT	TCTGTTGCTG	4020
15	CTTTATTTTCG	ACCCCAGAGC	TCTGGTCTGT	GTGCTTTCAT	GTCGCTGCTT	TATTAAATCT	4080
	TACCTTCTAC	ATTTTATGTA	TGGTCTCAGT	GTCTTCTTGG	GTACGCGGCT	GTCCCGGGAC	4140
20	TTGAGTGTCT	GAGTGAGGGT	CTTCCCTCGA	GGGTCTTTCA	TTTGGTACAT	GGGCCGGGAA	4200
	TTCGAGAATC	TTTCATTTGG	TGCATTGGCC	GGGAATTCGA	AAATCTTTCA	GATCCCCGGG	4260
	TACCGAGCTC	GAATTCCGGT	CTCCCTATAG	TGAGTCGTAT	TAATTTTCGAT	AAGCCAGCTG	4320
25	CATTAATGAA	TCGGCCAACG	CGCGGGGAGA	GGCGGTTTGC	GTATTGGGCG	CTCTTCCGCT	4380
	TCCTCGCTCA	CTGACTCGCT	GCGCTCGGTC	GTTGCGCTGC	GGCGAGCGGT	ATCAGCTCAC	4440
30	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	TCAGGGGATA	ACGCAGGAAA	GAACATGTGA	4500
	GCAAAAGGCC	AGCAAAAGGC	CAGGAACCGT	AAAAAGGCCG	CGTTGCTGGC	GTTTTTCCAT	4560
	AGGCTCCGCC	CCCCTGACGA	GCATCACAAA	AATCGACGCT	CAAGTCAGAG	GTGGCGAAAC	4620
35	CCGACAGGAC	TATAAAGATA	CCAGGCGTTT	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	4680
	GTTCCGACCC	TGCCGCTTAC	CGGATACCTG	TCCGCCTTTC	TCCCTTCGGG	AAGCGTGGCG	4740
	CTTTCTCATA	GCTCACGCTG	TAGGTATCTC	AGTTCGGTGT	AGGTCGTTCG	CTCCAAGCTG	4800
40	GGCTGTGTGC	ACGAACCCCC	CGTTCAGCCC	GACCGCTGCG	CCTTATCCGG	TAACATATCGT	4860
	CTTGAGTCCA	ACCCGGTAAG	ACACGACTTA	TCGCCACTGG	CAGCAGCCAC	TGGTAACAGG	4920
45	ATTAGCAGAG	CGAGGTATGT	AGGCGGTGCT	ACAGAGTTCT	TGAAGTGGTG	GCCTAACTAC	4980
	GGCTACACTA	GAAGGACAGT	ATTTGGTATC	TGCGCTCTGC	TGAAGCCAGT	TACCTTCGGA	5040
	AAAAGAGTTG	GTAGCTCTTG	ATCCGGCAAA	CAAACCACCG	CTGGTAGCGG	TGGTTTTTTT	5100
50	GTTTGCAAGC	AGCAGATTAC	GCGCAGAAAA	AAAGGATCTC	AAGAAGATCC	TTTGATCTTT	5160
	TCTACGGGGT	CTGACGCTCA	GTGGAACGAA	AACTCACGTT	AAGGGATTTT	GGTCATGAGA	5220
55	TTATCAAAAA	GGATCTTCAC	CTAGATCCTT	TTAAATTAAA	AATGAAGTTT	TAAATCAATC	5280
	TAAAGTATAT	ATGAGTAAAC	TTGGTCTGAC	AGTTACCAAT	GCTTAATCAG	TGAGGCACCT	5340
	ATCTCAGCGA	TCTGTCTATT	TCGTTCATCC	ATAGTTGCCT	GACTCCCCGT	CGTGTAGATA	5400
60	ACTACGATAC	GGGAGGGGCTT	ACCATCTGGC	CCCAGTGCTG	CAATGATACC	GCGAGACCCA	5460
	CGCTCACCGG	CTCCAGATTT	ATCAGCAATA	AACCAGCCAG	CCGGAAGGGC	CGAGCGCAGA	5520
65	AGTGGTCCTG	CAACTTTATC	CGCCTCCATC	CAGTCTATTA	ATTGTTGCCG	GGAAGCTAGA	5580

GTAAGTAGTT CGCCAGTTAA TAGTTTGC GC AACGTTGTTG CCATTGCTAC AGGCATCGTG 5640  
GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA 5700  
5 GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT 5760  
GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT 5820  
CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA 5880  
10 TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT 5940  
ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG GAAAACGTTT TCGGGGCGA 6000  
15 AAACCTCTCA GGATCTTACC GCTGTTGAGA TCCAGTTTCA TGTAACCCAC TCGTGCACCC 6060  
AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG 6120  
CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC 6180  
20 CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTATTGTG TCATGAGCGG ATACATATTT 6240  
GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA 6300  
25 CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG 6360  
AGGCCCTTTC GTCTCGCGCG TTTCCGGTGAT GACGGTGAAA ACCTCTGACA CATGCAGCTC 6420  
CCGGAGACGG TCACAGCTTG TCTGTAAGCG GATGCCGGGA GCAGACAAGC CCGTCAGGGC 6480  
30 GCGTCAGCGG GTGTTGGCGG GTGTCGGGGC TGGCTTAACT ATGCGGCATC AGAGCAGATT 6540  
GTACTGAGAG TGCACCATAT CGACGCTCTC CCTTATGCGA CTCCTGCATT AGGAAGCAGC 6600  
CCAGTAGTAG GTTGAGGCCG TTGAGCACCG CCGCCGCAAG GAATGGTGCA AGGAGATGGC 6660  
35 GCCCAACAGT CCCCCGGCCA CGGGGCTGTC CACCATACCC ACGCCGAAAC AAGCGCTCAT 6720  
GAGCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT AGGCGCCAGC 6780  
40 AACC GCACCT GTGGCGCCGG TGATGCCGGC CACGATGCGT CCGGCGTAGA GGATCTGGCT 6840  
AGCGATGACC CTGCTGATTG GTTCGCTGAC CATTTCCGGG GTGCGGAACG GCGTTACCAG 6900  
AAACTCAGAA GGTTCTGTCCA ACCAAACCGA CTCTGACGGC AGTTTACGAG AGAGATGATA 6960  
45 GGGTCTGCTT CAGTAAGCCA GATGCTACAC AATTAGGCTT GTACATATTG TCGTTAGAAC 7020  
GCGGCTACAA TTAATACATA ACCTTATGTA TCATACACAT ACGATTTAGG TGACACTATA 7080

50

(2) INFORMATION FOR SEQ ID NO:22:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6795 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: DNA (genomic)

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATGAAAGAC CCCACCTGTA GGTGTTGGCAA GCTAGCTTAA GTAACGCCAT TTTGCAAGGC

60

	ATGGAAAAAT	ACATAACTGA	GAATAGAGAA	GTTTCAGATCA	AGGTCAGGAA	CAGATGGAAC	120
	AGCTGAATAT	GGGCCAAACA	GGATATCTGT	GGTAAGCAGT	TCCTGCCCCG	GCTCAGGGCC	180
5	AAGAACAGAT	GGAACAGCTG	AATATGGGCC	AAACAGGATA	TCTGTGGTAA	GCAGTTCCTG	240
	CCCCGGCTCA	GGGCCAAGAA	CAGATGGTCC	CCAGATGCGG	TCCAGCCCTC	AGCAGTTTCT	300
10	AGAGAACCAT	CAGATGTTTC	CAGGGTGCCC	CAAGGACCTG	AAATGACCCCT	GTGCCTTATT	360
	TGAACTAACC	AATCAGTTCG	CTTCTCGCTT	CTGTTGCGCG	GCTTCTGCTC	CCCGAGCTCA	420
	ATAAAAGAGC	CCACAACCCC	TCACTCGGGG	CGCCAGTCCT	CCGATTGACT	GAGTCGCCCCG	480
15	GGTACCCGTG	TATCCAATAA	ACCCTCTTGC	AGTTGCATCC	GACTTGTGGT	CTCGCTGTTC	540
	CTTGGGAGGG	TCTCCTCTGA	GTGATTGACT	ACCCGTCAGC	GGGGGTCTTT	CATTTGGGGG	600
20	CTCGTCCGGG	ATCGGGAGAC	CCCTGCCCAG	GGACCACCGA	CCCACCACCG	GGAGGTAAGC	660
	TGGCCAGCAA	CTTATCTGTG	TCTGTCCGAT	TGTCTAGTGT	CTATGACTGA	TTTTATGCGC	720
	CTGCGTCGGT	ACTAGTTAGC	TAAGTAGCTC	TGTATCTGGC	GGACCCGTGG	TGGAAGTGAC	780
25	GAGTTCGGAA	CACCCGGCCG	CAACCCTGGG	AGACGTCCCA	GGAGGAACAG	GGGAGGATCA	840
	GGGACGCCTG	GTGGACCCCT	TTGAAGGCCA	AGAGACCATT	TGGGGTTGCG	AGATCGTGGG	900
30	TTTCGAGTCCC	ACCTCGTGCC	CAGTTGCGAG	ATCGTGGGTT	CGAGTCCCAC	CTCGTGTTTT	960
	GTTGCGAGAT	CGTGGGTTCG	AGTCCACCT	CGCGTCTGGT	CACGGGATCG	TGGGTTTCGAG	1020
	TCCCACCTCG	TGTTTTGTTC	CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	TCTGGTCACG	1080
35	GGATCGTGGG	TTTCGAGTCCC	ACCTCGTGCA	GAGGGTCTCA	ATTGGCCGGC	CTTAGAGAGG	1140
	CCATCTGATT	CTTCTGGTTT	CTCTTTTTGT	CTTAGTCTCG	TGTCCGCTCT	TGTTGTGACT	1200
	ACTGTTTTTC	TAAAAATGGG	ACAATCTGTG	TCCACTCCCC	TTTCTCTGAC	TCTGGTTCTG	1260
40	TCGCTTGTA	ATTTTGTTC	TTTACGTTT	TTTTTGTGAG	TCGTCTATGT	TGTCTGTTAC	1320
	TATCTTGTTT	TTGTTTGTGG	TTTACGGTTT	CTGTGTGTGT	CTTGTGTGTC	TCTTTGTGTT	1380
45	CAGACTTGGA	CTGATGACTG	ACGACTGTTT	TTAAGTTATG	CCTTCTAAAA	TAAGCCTAAA	1440
	AATCCTGTCA	GATCCCTATG	CTGACCACTT	CCTTTCAGAT	CAACAGCTGC	CCTTACTCGA	1500
	GCTCAAGCTT	CGAATTCTGC	AGTCGACGGT	ACCGCGGCCG	CTAACTAATA	GCCCATTCTC	1560
50	CAAGGTACGT	AGCGGGGATC	AATTCCGCCC	CCCCCCTAAC	GTTACTGGCC	GAAGCCGCTT	1620
	GGAATAAGGC	CGGTGTGCGT	TTGTCTATAT	GTTATTTTCC	ACCATATTGC	CGTCTTTTGG	1680
55	CAATGTGAGG	GCCCCGAAAC	CTGGCCCTGT	CTTCTTGACG	AGCATTCCTA	GGGGTCTTTC	1740
	CCCTCTCGCC	AAAGGAATGC	AAGGTCTGTT	GAATGTCGTG	AAGGAAGCAG	TTCTCTGGA	1800
	AGCTTCTTGA	AGACAAACAA	CGTCTGTAGC	GACCCTTTGC	AGGCAGCGGA	ACCCCCACC	1860
60	TGGCGACAGG	TGCCCTCTGCG	GCCAAAAGCC	ACGTGTATAA	GATACACCTG	CAAAGGCGGC	1920
	ACAACCCAG	TGCCACGTTG	TGAGTTGGAT	AGTTGTGGAA	AGAGTCAAAT	GGCTCTCCTC	1980
65	AAGCGTATTC	AACAAGGGGC	TGAAGGATGC	CCAGAAGGTA	CCCATTGTA	TGGGATCTGA	2040

	TCTGGGGCCT	CGGTGCACAT	GCTTTACATG	TGTTTAGTCG	AGGTAAAAA	AACGTCTAGG	2100
	CCCCCGAAC	CACGGGGACG	TGGTTTTCCCT	TTGAAAAACA	CGATACGGGA	TCCACCGGTC	2160
5	GCCACCATGG	GTAAAGGAGA	AGAACTTTTC	ACAGGAGTTG	TCCCAATTCT	TGTTGAATTA	2220
	GATGGTGATG	TTAATGGGCA	CAAATTTTCT	GTCAGTGGAG	AGGGTGAAGG	TGATGCAACA	2280
10	TACGGAAAAC	TTACCCTTAA	ATTTATTTGC	ACTACTGGAA	AACTACCTGT	TCCATGGCCA	2340
	ACACTTGTCA	CTACTTTCAC	TTATGGTGTT	CAATGCTTTT	CAAGATACCC	AGATCATATG	2400
	AAACGGCATG	ACTTTTTCAA	GAGTGCCATG	CCCGAAGGTT	ATGTACAGGA	AAGAACTATA	2460
15	TTTTTCAAAG	ATGACGGGAA	CTACAAGACA	CGTGCTGAAG	TCAAGTTTGA	AGGTGATACC	2520
	CTTGTTAATA	GAATCGAGTT	AAAAGGTATT	GATTTTAAAG	AAGATGGAAA	CATTCTTGGA	2580
20	CACAAATTGG	AATACAATA	TAACTCACAC	AATGTATACA	TCATGGCAGA	CAACAAAAAG	2640
	AATGGAACCA	AAGTTAACTT	CAAATTAGA	CACAACATTG	AAGATGGAAG	CGTTCAACTA	2700
	GCAGACCATT	ATCAACAAAA	TACTCCAATT	GGCGATGGCC	CTGTCCTTTT	ACCAGACAAC	2760
25	CATTACCTGT	CCACACAATC	TGCCCTTTTCG	AAAGATCCCA	ACGAAAAGAG	AGACCACATG	2820
	GTCCTTCTTG	AGTTTGTAAC	AGCTGCTGGG	ATTACACATG	GCATGGATGA	ACTATACAAG	2880
	TCCGGATCTA	GATAACTGTA	TCGATGGATC	CGAAGGCGGG	GACAGCAGTG	CAGTGGTGGA	2940
30	CAGAAAGCAA	GTGATCTAGG	CCAGCAGCCT	CCCTAAAGGG	ACTTCAGCCC	ACAAAGCCAA	3000
	ACTTGTGGCT	TTAATACAAG	CTCTGTAAAT	GGTAAAAAAA	AAAAAGTCTA	CACGGACAGC	3060
35	AGGTATGCTC	TTGCCACTGT	ACAGAGCAAT	ATACAGACAA	AGAGAACTGT	TGACATCTGC	3120
	AGAGAAAGAC	CTAAGATGCT	GTGGCTAAAA	GAAATCAGAT	GGCAAATCTA	ACCGCCCAGG	3180
	CATCCTAAAG	AGCAATGATC	CTGACAGTCT	GAAGACTATC	AAGTTATAGA	CAAATTAAGA	3240
40	CTGGTAAAAA	AAACCCTGTA	TAAAATAGTA	AAAAGTAAAA	AAAGAAACT	AGTCCTCTCA	3300
	TGAGAAGACA	GACCTGACAT	CTACTGAAAA	ATAGACTTTA	CTGGAAAAAA	TATGTGTATG	3360
45	AATACCTTCT	AGTTTTTGTG	AACGTTCTCA	AGATGGATAA	AAGCTTTTCC	TTGTAAAACG	3420
	AGACTGATCA	GATAGTCATC	AAGAAGATTG	TTAAAGAAAA	TTTTCCAAGG	TTCGGAGTGC	3480
	CAAAAGCAAT	AGTGTCAGAT	AATGGTCCTG	CCTTTGTTGC	CCAGGTAAGT	CAGGGTGTGG	3540
50	CCAAGTATTT	AGAGGTCAAA	TGAAAATTCC	ATTGTGTGTA	CAGACCTCAG	AGCTCAGGAA	3600
	AGATAAAAAA	GAATAAATAA	AACTCTAAAC	AGACCTTGAC	AAAATTAATC	CTAGAGACTG	3660
55	GCACAGACTT	ACTTGGTACT	CCTTCCCCTT	GCCCTATTTA	GAAGTGAAG	TACTCCCTCT	3720
	TGATTCGGTT	TTACTCTTTT	TAAGATCCTT	TATGGGGCTC	CTATGCCATC	ACTGTCTTAA	3780
	ATGATGTGTT	TAAACCTATG	TTGTTATAAT	AATGATCTAT	ATGTTAAGTT	AAAAGGCTTG	3840
60	CAGGTGGTGC	AGAAAGAAGT	CTGGTCACAA	CTGGCTACAG	TGAACAAGCT	GGGTACCCCA	3900
	AGGACATCTT	ACCAGTTCCA	GCCAGAGATC	TGATCTACGA	TCCCCGGGTC	GACCCGGGTC	3960
65	GACCCTGTGG	AATGTGTGTC	AGTTAGGGTG	TGGAAAGTCC	CCAGGCTCCC	CAGCAGGCAG	4020
	AAGTATGCAA	AGCATGCATC	TCAATTAGTC	AGCAACCAGG	TGTGGAAAGT	CCCCAGGCTC	4080

	CCCAGCAGGC	AGAAGTATGC	AAAGCATGCA	TCTCAATTAG	TCAGCAACCA	TAGTCCCGCC	4140
5	CCTAACTCCG	CCCATCCCGC	CCCTAACTCC	GCCCAGTTCC	GCCCATTTCTC	CGCCCCATGG	4200
	CTGACTAATT	TTTTTTATTT	ATGCAGAGGC	CGAGGCCGCC	TCGGCCTCTG	AGCTATTCCA	4260
	GAAGTAGTGA	GGAGGCTTTT	TTGGAGGCCT	AGGCTTTTGC	AAAAAGCTTC	ACGCTGCCGC	4320
10	AAGCACTCAG	GGCGCAAGGG	CTGCTAAAGG	AAGCGGAACA	CGTAGAAAGC	CAGTCCGCAG	4380
	AAACGGTGCT	GACCCCGGAT	GAATGTCAGC	TACTGGGCTA	TCTGGACAAG	GGAAAACGCA	4440
15	AGCGCAAAGA	GAAAGCAGGT	AGCTTGCACT	GGGCTTACAT	GGCGATAGCT	AGACTGGGCG	4500
	GTTTTATGGA	CAGCAAGCGA	ACCGGAATTG	CCAGCTGGGG	CGCCCTCTGG	TAAGGTTGGG	4560
	AAGCCCTGCA	AAGTAAACTG	GATGGCTTTC	TTGCCGCCAA	GGATCTGATG	GCGCAGGGGA	4620
20	TCAAGATCTG	ATCAAGAGAC	AGGATGAGGA	TCGTTTCGCA	TGATTGAACA	AGATGGATTG	4680
	CACGCAGGTT	CTCCGGCCGC	TTGGGTGGAG	AGGCTATTTC	GCTATGACTG	GGCACAACAG	4740
25	ACAATCGGCT	GCTCTGATGC	CGCCGTGTTT	CGGCTGTCAG	CGCAGGGGCG	CCCGGTTCTT	4800
	TTTGTCAAGA	CCGACCTGTC	CGGTGCCCTG	AATGAACTGC	AGGACGAGGC	AGCGCGGCTA	4860
	TCGTGGCTGG	CCACGACGGG	CGTTCCCTTG	GCAGCTGTGC	TCGACGTTGT	CACTGAAGCG	4920
30	GGAAGGGACT	GGCTGCTATT	GGGCGAAGTG	CCGGGGCAGG	ATCTCCTGTC	ATCTCACCTT	4980
	GCTCCTGCCG	AGAAAGTATC	CATCATGGCT	GATGCAATGC	GGCGGCTGCA	TACGCTTGAT	5040
	CCGGCTACCT	GCCCATTCTG	CCACCAAGCG	AAACATCGCA	TCGAGCGAGC	ACGTACTCGG	5100
35	ATGGAAGCCG	GTCTTGTCGA	TCAGGATGAT	CTGGACGAAG	AGCATCAGGG	GCTCGCGCCA	5160
	GCCGAAGTGT	TCGCCAGGCT	CAAGGCGCGC	ATGCCCGACG	GCGAGGATCT	CGTCGTGACC	5220
40	CATGGCGATG	CCTGCTTGCC	GAATATCATG	GTGGAAAATG	GCCGCTTTTC	TGGATTTCATC	5280
	GACTGTGGCC	GGCTGGGTGT	GGCGGACCGC	TATCAGGACA	TAGCGTTGGC	TACCCGTGAT	5340
	ATTGCTGAAG	AGCTTGGCGG	CGAATGGGCT	GACCGCTTCC	TCGTGCTTTA	CGGTATCGCC	5400
45	GCTCCCGATT	CGCAGCGCAT	CGCCTTCTAT	CGCCTTCTTG	ACGAGTTCTT	CTGAGCGGGA	5460
	CTCTGGGGTT	CGAAATGACC	GACCAAGCGA	CGCCCAACCT	GCCATCACGA	GATTTTCGATT	5520
50	CCACCGCCGC	CTTCTATGAA	AGGTTGGGCT	TCGGAATCGT	TTTCCGGGAC	GGAATTCGTA	5580
	ATCTGCTGCT	TGCAAAACAAA	AAAACCACCG	CTACCAGCGG	TGGTTTGTTT	GCCGGATCAA	5640
	GAGCTACCAA	CTCTTTTTTC	GAAGGTAAGT	GGCTTCAGCA	GAGCGCAGAT	ACCAAATACT	5700
55	GTCCTTCTAG	TGTAGCCGTA	GTTAGGCCAC	CACTTCAAGA	ACTCTGTAGC	ACCGCCTACA	5760
	TACCTCGCTC	TGCTAATCCT	GTTACCACTG	GCTGCTGCCA	GTGGCGATAA	GTCGTGTCTT	5820
60	ACCGGGTTGG	ACTCAAGACG	ATAGTTACCG	GATAAGGCGC	AGCGGTCGGG	CTGAACGGGG	5880
	GGTTTCGTGCA	CACAGCCCGA	CTTGAGCGA	ACGACCTACA	CCGAAGTGA	ATACCTACAG	5940
	CGTGAGCATT	GAGAAAGCGC	CACGCTTCCC	GAAGGGAGAA	AGGCGGACAG	GTATCCGGTA	6000
65	AGCGGCAGGG	TCGGAACAGG	AGAGCGCACG	AGGGAGCTTC	CAGGGGGAAA	CGCCTGGTAT	6060

	CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGA	CTTGTGAGC GTCGATTTTT GTGATGCTCG	6120
	TCAGGGGGGC GGAGCCTATG GAAAAACGCC	AGCAACGCCG AGATGCGCCG CCTCGAGAAC	6180
5	CCTGGCCCTA TTATTGGGTG GACTAACCAT	GGGGGGAATT GCCGCTGGAA TAGGAACAGG	6240
	GACTACTGCT CTAATGGCCA CTCAGCAATT	CCAGCAGCTC CAAGCCGCAG TACAGGATGA	6300
10	TCTCAGGGAG GTTGAAAAAT CAATCTCTAA	CCTAGAAAAG TCTCTCACTT CCCTGTCTGA	6360
	AGTTGTCCTA CAGAATCGAA GGGGCCTAGA	CTTGTTATTT CTAAAAGAAG GAGGGCTGTG	6420
	TGCTGCTCTA AAAGAAGAAT GTTGCTTCTA	TGCGGACCAC ACAGGACTAG TGAGAGACAG	6480
15	CATGGCCAAA TTGAGAGAGA GGCTTAATCA	GAGACAGAAA CTGTTTGAGT CAACTCAAGG	6540
	ATGGTTTGAG GGACTGTTTA ACAGATCCCC	TTGGTTTACC ACCTTGATAT CTACCATTAT	6600
20	GGGACCCCTC ATTGTACTCC TAATGATTTT	GCTCTTCGGA CCCTGCATTC TTAATCGATT	6660
	AGTCCAATTT GTTAAAGACA GGATATCAGT	GGTCCAGGCT CTAGTTTTGA CTCAACAATA	6720
	TCACCAGCTG AAGCCTATAG AGTACGAGCC	ATAGATAAAA TAAAAGATTT TATTTAGTCT	6780
25	CCAGAAAAAG GGGGG		6795
	(2) INFORMATION FOR SEQ ID NO:23:		
	(i) SEQUENCE CHARACTERISTICS:		
30	(A) LENGTH: 9093 base pairs		
	(B) TYPE: nucleic acid		
	(C) STRANDEDNESS: single		
	(D) TOPOLOGY: linear		
35	(ii) MOLECULE TYPE: DNA (genomic)		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:		
40	AATGAAAGAC CCCACCTGTA GGT	TTGGCAA GCTAGCTTAA GTAACGCCAT TTTGCAAGGC	60
	ATGGAAAAAT ACATAACTGA GA	ATAGAGAA GTTCAGATCA AGGTCAGGAA CAGATGGAAC	120
45	AGCTGAATAT GGGCCAAACA GG	ATATCTGT GGTAAGCAGT TCCTGCCCCG GCTCAGGGCC	180
	AAGAACAGAT GGAACAGCTG A	ATATGGGCC AAACAGGATA TCTGTGGTAA GCAGTTCCTG	240
50	CCCCGGCTCA GGGCCAAGAA C	AGATGGTCC CCAGATGCGG TCCAGCCCTC AGCAGTTTCT	300
	AGAGAACCAT CAGATGTTTC C	AGGGTGCCC CAAGGACCTG AAATGACCCT GTGCCTTATT	360
	TGAACTAACC AATCAGTTCG C	TTCTCGCTT CTGTTCGCGC GCTTCTGCTC CCCGAGCTCA	420
55	ATAAAAGAGC CCACAACCCC T	ACTCGGGG CGCCAGTCCT CCGATTGACT GAGTCGCCCCG	480
	GGTACCCGTG TATCCAATAA A	CCCTCTTGC AGTTGCATCC GACTTGTGGT CTCGCTGTTC	540
60	CTTGGGAGGG TCTCCTCTGA G	TGATTGACT ACCCGTCAGC GGGGGTCTTT CATTTGGGGG	600
	CTCGTCCGGG ATCGGGAGAC C	CTGCCCAG GGACCACCGA CCCACCACCG GGAGGTAAGC	660
	TGGCCAGCAA CTTATCTGTG T	CTGTCCGAT TGTCTAGTGT CTATGACTGA TTTTATGCGC	720
65	CTGCGTCGGT ACTAGTTAGC T	AACTAGCTC TGTATCTGGC GGACCCGTGG TGGA	780

	GAGTTCGGAA	CACCCGGCCG	CAACCCTGGG	AGACGTCCCA	GGAGGAACAG	GGGAGGATCA	840
	GGGACGCCTG	GTGGACCCCT	TTGAAGGCCA	AGAGACCATT	TGGGGTTGCG	AGATCGTGGG	900
5	TTTCGAGTCCC	ACCTCGTGCC	CAGTTGCGAG	ATCGTGGGTT	CGAGTCCCAC	CTCGTGTTTT	960
	GTTGCGAGAT	CGTGGGTTCG	AGTCCCACCT	CGCGTCTGGT	CACGGGATCG	TGGGTTCGAG	1020
10	TCCCACCTCG	TGTTTTGTTC	CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	TCTGGTCACG	1080
	GGATCGTGGG	TTTCGAGTCCC	ACCTCGTGCA	GAGGGTCTCA	ATTGGCCGGC	CTTAGAGAGG	1140
	CCATCTGATT	CTTCTGGTTT	CTCTTTTGT	CTTAGTCTCG	TGTCCGCTCT	TGTTGTGACT	1200
15	ACTGTTTTTC	TAAAAATGGG	ACAATCTGTG	TCCACTCCCC	TTTCTCTGAC	TCTGGTTCTG	1260
	TCGCTTGGTA	ATTTTGTTC	TTTACGTTT	TTTTTGTGAG	TCGTCTATGT	TGTCTGTTAC	1320
20	TATCTTGTTT	TTGTTTGTGG	TTTACGGTTT	CTGTGTGTGT	CTTGTGTGTC	TCTTTGTGTT	1380
	CAGACTTGGA	CTGATGACTG	ACGACTGTTT	TTAAGTTATG	CCTTCTAAAA	TAAGCCTAAA	1440
	AATCCTGTCA	GATCCCTATG	CTGACCACTT	CCTTTCAGAT	CAACAGCTGC	CCTTACGTAT	1500
25	CGATGGATCC	CTCGACTAAC	TAATAGCCCA	TTCTCCAAGG	TCGAGCGGGA	TCAATTCCGC	1560
	CCCCCCCCCTA	ACGTTACTGG	CCGAAGCCGC	TTGGAATAAG	GCCGGTGTGC	GTTTGTCTAT	1620
30	ATGTTATTTT	CCACCATATT	GCCGTCTTTT	GGCAATGTGA	GGGCCCAGAA	ACCTGGCCCT	1680
	GTCTTCTTGA	CGAGCATTC	TAGGGGTCTT	TCCCCTCTCG	CCAAAGGAAT	GCAAGGTCTG	1740
	TTGAATGTCG	TGAAGGAAGC	AGTTCCTCTG	GAAGCTTCTT	GAAGACAAAC	AACGTCTGTA	1800
35	GCGACCCCTT	GCAGGCAGCG	GAACCCCCCA	CCTGGCGACA	GGTGCCTCTG	CGGCCAAAAG	1860
	CCACGTGTAT	AAGATACACC	TGCAAAGGCG	GCACAACCCC	AGTGCCACGT	TGTGAGTTGG	1920
40	ATAGTTGTGG	AAAGAGTCAA	ATGGCTCTCC	TCAAGCGTAT	TCAACAAGGG	GCTGAAGGAT	1980
	GCCCAGAAGG	TACCCCATTC	TATGGGATCT	GATCTGGGGC	CTCGGTGCAC	ATGCTTTACA	2040
	TGTGTTTAGT	CGAGGTTAAA	AAAACGTCTA	GGCCCCCGA	ACCACGGGGA	CGTGGTTTTT	2100
45	CTTTGAAAAA	CACGATAATA	ATCATGGGCG	CGGATCCCGT	CGTTTTACAA	CGTCGTGACT	2160
	GGGAAAACCC	TGGCGTTACC	CAACTTAATC	GCCTTGACAG	ACATCCCCCT	TTGCCAGCT	2220
50	GGCGTAATAG	CGAAGAGGCC	CGCACCGATC	GCCCTTCCCA	ACAGTTGCGC	AGCCTGAATG	2280
	GCGAATGGCG	CTTTGCCTGG	TTTCCGGCAC	CAGAAGCGGT	GCCGGAAAGC	TGGCTGGAGT	2340
	GCGATCTTCC	TGAGGCCGAT	ACTGTCGTCG	TCCCCTCAAA	CTGGCAGATG	CACGGTTACG	2400
55	ATGCGCCCAT	CTACACCAAC	GTAACCTATC	CCATTACGGT	CAATCCGCCG	TTTGTTCCTA	2460
	CGGAGAATCC	GACGGGTTGT	TACTCGCTCA	CATTTAATGT	TGATGAAAGC	TGGCTACAGG	2520
60	AAGGCCAGAC	GCGAATTATT	TTTGATGGCG	TTAACTCGGC	GTTTCATCTG	TGGTGCAACG	2580
	GGCGCTGGGT	CGGTTACGGC	CAGGACAGTC	GTTTGCCGTC	TGAATTTGAC	CTGAGCGCAT	2640
	TTTACGCGC	CGGAGAAAAC	CGCCTCGCGG	TGATGGTGCT	GCGTTGGAGT	GACGGCAGTT	2700
65	ATCTGGAAGA	TCAGGATATG	TGGCGGATGA	GCGGCATTTT	CCGTGACGTC	TCGTTGCTGC	2760

	ATAAACCGAC	TACACAAATC	AGCGATTTCC	ATGTTGCCAC	TCGCTTTAAT	GATGATTTCA	2820
	GCCGCGCTGT	ACTGGAGGCT	GAAGTTCAGA	TGTGCGGCGA	GTTGCGTGAC	TACCTACGGG	2880
5	TAACAGTTTC	TTTATGGCAG	GGTGAACGC	AGGTCGCCAG	CGGCACCGCG	CCTTTCGGCG	2940
	GTGAAATTAT	CGATGAGCGT	GGTGGTTATG	CCGATCGCGT	CACACTACGT	CTGAACGTCG	3000
	AAAACCCGAA	ACTGTGGAGC	GCCGAAATCC	CGAATCTCTA	TCGTGCGGTG	GTTGAACTGC	3060
10	ACACCGCCGA	CGGCACGCTG	ATTGAAGCAG	AAGCCTGCGA	TGTCGGTTTC	CGCGAGGTGC	3120
	GGATTGAAAA	TGGTCTGCTG	CTGCTGAACG	GCAAGCCGTT	GCTGATTCGA	GGCGTTAACC	3180
15	GTCACGAGCA	TCATCCTCTG	CATGGTCAGG	TCATGGATGA	GCAGACGATG	GTGCAGGATA	3240
	TCCTGCTGAT	GAAGCAGAAC	AACTTTAACG	CCGTGCGCTG	TTCGCATTAT	CCGAACCATC	3300
	CGCTGTGGTA	CACGCTGTGC	GACCGCTACG	GCCTGTATGT	GGTGGATGAA	GCCAATATTG	3360
20	AAACCCACGG	CATGGTGCCA	ATGAATCGTC	TGACCGATGA	TCCGCGCTGG	CTACCGGCGA	3420
	TGAGCGAACG	CGTAACGCGA	ATGGTGCAGC	GCGATCGTAA	TCACCCGAGT	GTGATCATCT	3480
25	GGTCGCTGGG	GAATGAATCA	GGCCACGGCG	CTAATCACGA	CGCGCTGTAT	CGCTGGATCA	3540
	AATCTGTCTGA	TCCTTCCCCG	CCGGTGCAGT	ATGAAGGCGG	CGGAGCCGAC	ACCACGGCCA	3600
	CCGATATTAT	TTGCCCCGATG	TACGCGCGCG	TGGATGAAGA	CCAGCCCTTC	CCGGCTGTGC	3660
30	CGAAATGGTC	CATCAAAAAA	TGGCTTTCGC	TACCTGGAGA	GACGCGCCCG	CTGATCCTTT	3720
	GCGAATACGC	CCACGCGATG	GGTAACAGTC	TTGGCGGTTT	CGCTAAATAC	TGGCAGGCGT	3780
	TTCGTCAGTA	TCCCCGTTTA	CAGGGCGGCT	TCGTCTGGGA	CTGGGTGGAT	CAGTCGCTGA	3840
35	TTAAATATGA	TGAAAACGGC	AACCCGTGGT	CGGCTTACGG	CGGTGATTTT	GGCGATACGC	3900
	CGAACGATCG	CCAGTTCTGT	ATGAACGGTC	TGGTCTTTGC	CGACCGCACG	CCGCATCCAG	3960
40	CGCTGACGGA	AGCAAAACAC	CAGCAGCAGT	TTTTCCAGTT	CCGTTTATCC	GGGCAAACCA	4020
	TCGAAGTGAC	CAGCGAATAC	CTGTTCCGTC	ATAGCGATAA	CGAGCTCCTG	CACTGGATGG	4080
	TGGCGCTGGA	TGGTAAGCCG	CTGGCAAGCG	GTGAAGTGCC	TCTGGATGTC	GCTCCACAAG	4140
45	GTAAACAGTT	GATTGAACTG	CCTGAACTAC	CGCAGCCGGA	GAGCGCCGGG	CAACTCTGGC	4200
	TCACAGTACG	CGTAGTGCAA	CCGAACGCGA	CCGCATGGTC	AGAAGCCGGG	CACATCAGCG	4260
50	CCTGGCAGCA	GTGGCGTCTG	GCGGAAAACC	TCAGTGTGAC	GCTCCCCGCC	GCGTCCCACG	4320
	CCATCCCGCA	TCTGACCACC	AGCGAAATGG	ATTTTTGCAT	CGAGCTGGGT	AATAAGCGTT	4380
	GGCAATTTAA	CCGCCAGTCA	GGCTTTCCTT	CACAGATGTG	GATTGGCGAT	AAAAACAAC	4440
55	TGCTGACGCC	GCTGCGCGAT	CAGTTCACCC	GTGCACCGCT	GGATAACGAC	ATTGGCGTAA	4500
	GTGAAGCGAC	CCGCATTGAC	CCTAACGCCT	GGGTGCAACG	CTGGAAGGCG	GCGGGCCATT	4560
60	ACCAGGCCGA	AGCAGCGTTG	TTGCAGTGCA	CGGCAGATAC	ACTTGCTGAT	GCGGTGCTGA	4620
	TTACGACCGC	TCACGCGTGG	CAGCATCAGG	GGAAAACCTT	ATTTATCAGC	CGGAAAACCT	4680
	ACCGGATTGA	TGGTAGTGGT	CAAATGGCGA	TTACCGTTGA	TGTTGAAGTG	GCGAGCGATA	4740
65	CACCGCATCC	GGCGCGGATT	GGCCTGAACT	GCCAGCTGGC	GCAGGTAGCA	GAGCGGGTAA	4800



	ACTGGCTCGG	ATTAGGGCCG	CAAGAAACT	ATCCCGACCG	CCTTACTGCC	GCCTGTTTTG	4860
5	ACCGCTGGGA	TCTGCCATTG	TCAGACATGT	ATACCCCGTA	CGTCTTCCCG	AGCGAAAACG	4920
	GTCTGCGCTG	CGGGACGCGC	GAATTGAATT	ATGGCCCACA	CCAGTGGCGC	GGCGACTTCC	4980
	AGTTCAACAT	CAGCCGCTAC	AGTCAACAGC	AACTGATGGA	AACCAGCCAT	CGCCATCTGC	5040
10	TGCACGCGGA	AGAAGGCACA	TGGCTGAATA	TCGACGGTTT	CCATATGGGG	ATTGGTGGCG	5100
	ACGACTCCTG	GAGCCCGTCA	GTATCGGCGG	AATTCCAGCT	GAGCGCCGGT	CGCTACCATT	5160
15	ACCAGTTGGT	CTGGTGTCAA	AAATAATAAT	AACCGGGCAG	GGGGGATCCG	AAGGCGGGGA	5220
	CAGCAGTGCA	GTGGTGGACA	GAAAGCAAGT	GATCTAGGCC	AGCAGCCTCC	CTAAAGGGAC	5280
	TTCAGCCCAC	AAAGCCAAAC	TTGTGGCTTT	AATACAAGCT	CTGTAAATGG	TAAAAAAAAA	5340
20	AAAGTCTACA	CGGACAGCAG	GTATGCTCTT	GCCACTGTAC	AGAGCAATAT	ACAGACAAAG	5400
	AGAACTGTTG	ACATCTGCAG	AGAAAGACCT	AAGATGCTGT	GGCTAAAAGA	AATCAGATGG	5460
	CAAATCTAAC	CGCCCAGGCA	TCCTAAAGAG	CAATGATCCT	GACAGTCTGA	AGACTATCAA	5520
25	GTTATAGACA	AATTAAGACT	GGTAAAAAAA	ACCCTGTATA	AAATAGTAAA	AACTGAAAAA	5580
	AGAAAACTAG	TCCTCTCATG	AGAAGACAGA	CCTGACATCT	ACTGAAAAAT	AGACTTTACT	5640
30	GGAAAAAATA	TGTGTATGAA	TACCTTCTAG	TTTTTGTGAA	CGTTCTCAAG	ATGGATAAAA	5700
	GCTTTTCCTT	GTAAACGAG	ACTGATCAGA	TAGTCATCAA	GAAGATTGTT	AAAGAAAATT	5760
	TTCCAAGGTT	CGGAGTGCCA	AAAGCAATAG	TGTCAGATAA	TGGTCCTGCC	TTTGTTGCCC	5820
35	AGGTAAGTCA	GGGTGTGGCC	AAGTATTTAG	AGGTCAAAATG	AAAATTCCAT	TGTGTGTACA	5880
	GACCTCAGAG	CTCAGGAAAG	ATAAAAAAGA	ATAAATAAAA	CTCTAAACAG	ACCTTGACAA	5940
40	AATTAATCCT	AGAGACTGGC	ACAGACTTAC	TTGGTACTCC	TTCCCCTTGC	CCTATTTAGA	6000
	ACTGAGAATA	CTCCCTCTTG	ATTCGGTTTT	ACTCTTTTTA	AGATCCTTTA	TGGGGCTCCT	6060
	ATGCCATCAC	TGTCTTAAAT	GATGTGTTTA	AACCTATGTT	GTTATAATAA	TGATCTATAT	6120
45	GTTAAGTTAA	AAGGCTTGCA	GGTGGTGCAG	AAAGAAGTCT	GGTCACAACT	GGCTACAGTG	6180
	AACAAGCTGG	GTACCCCAAG	GACATCTTAC	CAGTTCCAGC	CAGAGATCTG	ATCTACGATC	6240
50	CCCGGGTCGA	CCCGGGTCGA	CCCTGTGGAA	TGTGTGTCAG	TTAGGGTGTG	GAAAGTCCCC	6300
	AGGCTCCCCA	GCAGGCAGAA	GTATGCAAAG	CATGCATCTC	AATTAGTCAG	CAACCAGGTG	6360
	TGGAAAGTCC	CCAGGCTCCC	CAGCAGGCAG	AAGTATGCAA	AGCATGCATC	TCAATTAGTC	6420
55	AGCAACCATA	GTCCCGCCCC	TAATCCGCC	CATCCCGCCC	CTAACTCCGC	CCAGTTCCGC	6480
	CCATTCTCCG	CCCCATGGCT	GACTAATTTT	TTTTATTTAT	GCAGAGGCCG	AGGCCGCCTC	6540
60	GGCCTCTGAG	CTATTCCAGA	AGTAGTGAGG	AGGCTTTTTT	GGAGGCCTAG	GCTTTTGCAA	6600
	AAAGCTTCAC	GCTGCCGCAA	GCACTCAGGG	CGCAAGGGCT	GCTAAAGGAA	GCGGAACACG	6660
	TAGAAAGCCA	GTCCGCAGAA	ACGGTGCTGA	CCCCGGATGA	ATGTCAGCTA	CTGGGCTATC	6720
65	TGGACAAGGG	AAAACGCAAG	CGCAAAGAGA	AAGCAGGTAG	CTTGCAAGTG	GCTTACATGG	6780

	CGATAGCTAG	ACTGGGCGGT	TTTATGGACA	GCAAGCGAAC	CGGAATTGCC	AGCTGGGGCG	6840
	CCCTCTGGTA	AGGTTGGGAA	GCCCTGCAAA	GTAAACTGGA	TGGCTTTCTT	GCCGCCAAGG	6900
5	ATCTGATGGC	GCAGGGGATC	AAGATCTGAT	CAAGAGACAG	GATGAGGATC	GTTTCGCATG	6960
	ATTGAACAAG	ATGGATTGCA	CGCAGGTTCT	CCGGCCGCTT	GGGTGGAGAG	GCTATTCGGC	7020
10	TATGACTGGG	CACAACAGAC	AATCGGCTGC	TCTGATGCCG	CCGTGTTCCG	GCTGTCAGCG	7080
	CAGGGGCGCC	CGGTTCTTTT	TGTCAAGACC	GACCTGTCCG	GTGCCCTGAA	TGAACTGCAG	7140
	GACGAGGCAG	CGCGGCTATC	GTGGCTGGCC	ACGACGGGCG	TTCCTTGCGC	AGCTGTGCTC	7200
15	GACGTTGTCA	CTGAAGCGGG	AAGGGACTGG	CTGCTATTGG	GCGAAGTGCC	GGGGCAGGAT	7260
	CTCCTGTCA	CTCACCTTGC	TCCTGCCGAG	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG	7320
20	CGGCTGCATA	CGCTTGATCC	GGCTACCTGC	CCATTTCGACC	ACCAAGCGAA	ACATCGCATC	7380
	GAGCGAGCAC	GTACTCGGAT	GGAAGCCGGT	CTTGTCGATC	AGGATGATCT	GGACGAAGAG	7440
	CATCAGGGGC	TCGCGCCAGC	CGAACTGTTC	GCCAGGCTCA	AGGCGCGCAT	GCCCCACGGC	7500
25	GAGGATCTCG	TCGTGACCCA	TGGCGATGCC	TGCTTGCCGA	ATATCATGGT	GGAAAATGGC	7560
	CGCTTTTCTG	GATTCATCGA	CTGTGGCCGG	CTGGGTGTGG	CGGACCGCTA	TCAGGACATA	7620
	GCGTTGGCTA	CCCGTGATAT	TGCTGAAGAG	CTTGCGGGCG	AATGGGCTGA	CCGCTTCCTC	7680
30	GTGCTTTACG	GTATCGCCGC	TCCCGATTCT	CAGCGCATCG	CCTTCTATCG	CCTTCTTGAC	7740
	GAGTTCTTCT	GAGCGGGACT	CTGGGGTTCT	AAATGACCGA	CCAAGCGACG	CCCAACCTGC	7800
35	CATCACGAGA	TTTCGATTCC	ACCGCCGCCT	TCTATGAAAG	GTTGGGCTTC	GGAATCGTTT	7860
	TCCGGGACGG	AATTCGTAAT	CTGCTGCTTG	CAAACAAAAA	AACCACCGCT	ACCAGCGGTG	7920
	GTTTGTGTTGC	CGGATCAAGA	GCTACCAACT	CTTTTTCCTG	AGGTAAGTGG	CTTCAGCAGA	7980
40	GCGCAGATAC	CAAATACTGT	CCTTCTAGTG	TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	8040
	TCTGTAGCAC	CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	TGCTGCCAGT	8100
45	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	8160
	CGGTGCGGCT	GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	GACCTACACC	8220
	GAACTGAGAT	ACCTACAGCG	TGAGCATTGA	GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG	8280
50	GCGGACAGGT	ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	GGAGCTTCCA	8340
	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT	8400
55	CGATTTTTGT	GATGCTCGTC	AGGGGGGCGG	AGCCTATGGA	AAAACGCCAG	CAACGCCGAG	8460
	ATGCGCCGCC	TCGAGAACCC	TGGCCCTATT	ATTGGGTGGA	CTAACCATGG	GGGGAATTGC	8520
	CGCTGGAATA	GGAACAGGGA	CTACTGCTCT	AATGGCCACT	CAGCAATTCC	AGCAGCTCCA	8580
60	AGCCGCAGTA	CAGGATGATC	TCAGGGAGGT	TGAAAAATCA	ATCTCTAACC	TAGAAAAGTC	8640
	TCTCACTTCC	CTGTCTGAAG	TTGTCCTACA	GAATCGAAGG	GGCCTAGACT	TGTTATTTCT	8700
65	AAAAGAAGGA	GGGCTGTGTG	CTGCTCTAAA	AGAAGAATGT	TGCTTCTATG	CGGACCACAC	8760
	AGGACTAGTG	AGAGACAGCA	TGGCCAAATT	GAGAGAGAGG	CTTAATCAGA	GACAGAAACT	8820

GTTTGAGTCA ACTCAAGGAT GGTTCGAGGG ACTGTTTAAAC AGATCCCCTT GGTTTACCAC 8880  
 CTTGATATCT ACCATTATGG GACCCCTCAT TGTACTCCTA ATGATTTTGC TCTTCGGACC 8940  
 5 CTGCATTCTT AATCGATTAG TCCAATTTGT TAAAGACAGG ATATCAGTGG TCCAGGCTCT 9000  
 AGTTTTGACT CAACAATATC ACCAGCTGAA GCCTATAGAG TACGAGCCAT AGATAAAATA 9060  
 10 AAAGATTTTA TTTAGTCTCC AGAAAAAGGG GGG 9093

(2) INFORMATION FOR SEQ ID NO:24:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 46 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 20 (ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:  
 GACTAACCTT GATTCCCTGG AGGCGGGGGT CTTTCATTG GGGGCT 46

(2) INFORMATION FOR SEQ ID NO:25:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 4834 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 35 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: DNA (genomic)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:  
 TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC 60  
 45 CCCTCCCATC TGGAAAACAT ACTTGAGAAA AACATTTTCT GGAACAACCA CAGAATGTTT 120  
 CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG 180  
 50 TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCTTTA 240  
 AAAGCTTGTG AAAAATTTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG 300  
 AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTTTCGAC CCCAGAGCTC 360  
 55 TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CTTTCTACAT TTTATGTATG 420  
 GTCTCAGTGT CTTCTTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT 480  
 TCCCTCGAGG GTCTTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG 540  
 60 CATTGGCCGG GAATTCGAAA ATCTTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA 600  
 CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTTGT TCTGTTTTGG TCTGATGTCT 660  
 65 GTGTTCTGAT GTCTGTGTTT TGTCTCTAAG TCTGGTGCGA TCGCAGTTTC AGTTTTGCGG 720

	ACGCTCAGTG	AGACCGCGCT	CCGAGAGGGA	GTGCGGGGTG	GATAAGGATA	GACGTGTCCA	780
	GGTGTCCACC	GTCCGTTCGC	CCTGGGAGAC	GTCCCAGGAG	GAACAGGGGA	GGATCAGGGA	840
5	CGCCTGGTGG	ACCCCTTTGA	AGGCCAAGAG	ACCATTTGGG	GTTGCGAGAT	CGTGGGTTCTG	900
	AGTCCCACCT	CGTGCCCACT	TGCGAGATCG	TGGGTTCGAG	TCCCACCTCG	TGTTTTGTTC	960
10	CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	TCTGGTTCAG	GGATCGTGGG	TTCGAGTCCC	1020
	ACCTCGTGTT	TTGTTGCGAG	ATCGTGGGTT	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	1080
	CGTGGGTTCTG	AGTCCCACCT	CGTGCAGAGG	GTCTCAATTG	GCCGGCCTTA	GAGAGGCCAT	1140
15	CTGATTCTTC	TGGTTTCTCT	TTTTGTCTTA	GTCTCGTGTC	CGCTCTTGTT	GTGACTACTG	1200
	TTTTTCTAAA	AATGGGACAA	TCTGTGTCCA	CTCCCCTTTC	TCTGACTCTG	GTTCTGTCTG	1260
20	TTGGTAATTT	TGTTTGTTTA	CGTTTGTTTT	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	1320
	TTGTTTTTGT	TTGTGGTTTA	CGGTTTCTGT	GTGTGTCTTG	TGTGTCTCTT	TGTGTTTCTG	1380
	CTTGGAAGTA	TGACTGACGA	CTGTTTTTAA	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	1440
25	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTG	CCTCCCCTCT	1500
	CAACTCCAGA	GAGCAGCCAG	CGGGTCACAG	TGGTCCCGCC	CATGAACCTG	GAGCCTAGGG	1560
	AAAAATGAGC	TCGGAAATCC	GGAGCAAATG	AGGAGTGGTC	CCTGAGAAGT	CAGTGGCCTA	1620
30	AATGTTGTGG	CTGCTGAAGC	AAAAGAAGAG	GAGGCTGTTC	GAGTAGCCGG	CCAAGAGCGC	1680
	CGCGGGTTCC	CAGGCAGCTT	CTCATTCCCC	TGTCCCTCCC	ATCCCGTCTC	TTGTTAACAG	1740
35	AAAAACTGCT	TTCACCTTGA	GATATGAGTG	GCCCGATACA	GCCAGCTGTG	AGAGCTGTAC	1800
	TCCCTTCCCT	GCCCCACGTG	TTTTCTCTTC	TCAGGCGACC	CCTCCCTGAG	CTGCTGGCAG	1860
	TGAGTCTGTT	CTAAGCTCCA	GTGAGGGAGG	CATCCGCCCA	CTTGGGGCTT	CTGTCCAAGG	1920
40	TAAGGAGCAC	CTGTGAGTCT	AACTGCCAGG	CTCTGATGGG	GGTCTCGTCT	CTGTGGGACT	1980
	AGAAAGTGTC	CCAACAATCT	GACCAAGGTA	ACAGGAAGTT	AAGACAAAGA	CAGAGACCAA	2040
45	AGTCAGAATC	AGAGCTGTGC	TGTGAGACAA	AAAGATAAAA	AAAATAAAAT	GCTGGCCACA	2100
	AAAGTCAGGA	AAACTAGAAA	ACTTAGATAG	TACCTGGCAA	CAAAAGAAAG	CTTTTGGCTA	2160
	AAGATCAACG	TGTATACTGT	AAAGAAAATG	AGCACTGGGT	GAGAGACTGC	CCCAACAAAA	2220
50	AGAAGAGGAG	CCCCCTCAT	GACCAAACCC	TTCACCTGTT	CGTGGCTAAA	AGTAAAGAGA	2280
	TAACAAAAGG	GGTGCTAACA	CAGAAGCTGA	GTCTTAAAA	GAGTCCGGTG	GCCTACCTGT	2340
55	TGAAGCAGCT	AAAAAAGAGA	CTGTGTTTTCA	TACTCCTCCA	CTGACCAGTG	CAAAACAAGC	2400
	TAAAAAGTTC	CTGGGCACTG	CGGGCTTTTG	CAGATTGTGG	ATTCCAGGTT	TTGCTGAGTT	2460
	AAAGAGATAA	ACAGCCCTTC	GTATAGAAAA	ATAAAAAACA	ACCTTGATG	TCCTTGATG	2520
60	CTATTGAGAC	TGCCCTAATG	TTGTCCCCAG	CTATGGGACT	CCTAGATGTG	ACTGAGAACA	2580
	AAGGTATTGC	CAAAGAAGTT	CTTACTCAGA	GATTGGGACC	CTGAAAAAGA	CCTGTGGCAT	2640
65	ACTTGTAAGA	AATTAGACCT	GGTGGCTGTA	AGATGGCCTG	CTTGTCTGCA	CATAGTGGCT	2700
	TCTGGTCAAG	GACGCAGATA	AATTGACTCT	GAGACAAAAC	TTGGCACATG	TCCTAGAAAG	2760

	TGTGGTTCAG	CCCCCATGAC	CGATGGCTGA	CTAACGCTCT	TGAAAACATT	ATCCAACGTG	2820
5	TCCCCTGACC	GATGGACACA	TTGTCAGAGC	TTTTTTTGAC	TGAACGAGTG	ACCTTCGCTC	2880
	CCCCTGCTAT	CCTCGATCTC	ACTACTGCCT	GAGACTTCAC	CTACTCATCA	TTGTGCTGAC	2940
	ATTCTGGCAG	AAGAAACTCA	TACTCGAAAT	GATCTGAAGG	ATCAGATCAG	CCTTGGCCTG	3000
10	AGAGTTTGAG	CTGGTACACG	GATGGCAGTA	GCCTGGAGGT	TAAGGGTAAG	CGGAAGGCGG	3060
	GGACAGCAGT	GCAGTGGTGG	ACAGAAAGCA	AGTGATCTAG	GCCAGCAGCC	TCCCTAAAGG	3120
15	GACTTCAGCC	CACAAAGCCA	AACTTGTGGC	TTTAATACAA	GCTCTGTAAA	TGGTAAAAAA	3180
	AAAAAAGTCT	ACACGGACAG	CAGGTATGCT	CTTGCCACTG	TACAGAGCAA	TATACAGACA	3240
	AAGAGAACTG	TTGACATCTG	CAGAGAAAGA	CCTAAGATGC	TGTGGCTAAA	AGAAATCAGA	3300
20	TGGCAAATCT	AACCGCCAG	GCATCCTAAA	GAGCAATGAT	CCTGACAGTC	TGAAGACTAT	3360
	CAAGTTATAG	ACAAATTAAG	ACTGGTAAAA	AAAACCCTGT	ATAAAATAGT	AAAAACTGAA	3420
	AAAAGAAAAC	TAGTCCTCTC	ATGAGAAGAC	AGACCTGACA	TCTACTGAAA	AATAGACTTT	3480
25	ACTGGAAAAA	ATATGTGTAT	GAATACCTTC	TAGTTTTTGT	GAACGTTCTC	AAGATGGATA	3540
	AAAGCTTTTC	CTTGTA AAAAC	GAGACTGATC	AGATAGTCAT	CAAGAAGATT	GTTAAAGAAA	3600
	ATTTTCCAAG	GTTTCGGAGTG	CCAAAAGCAA	TAGTGT CAGA	TAATGGTCCT	GCCTTTGTTG	3660
30	CCCAGGTAAG	TCAGGGTGTG	GCCAAGTATT	TAGAGGTCAA	ATGAAAATTC	CATTGTGTGT	3720
	ACAGACCTCA	GAGCTCAGGA	AAGATAAAAA	AGAATAAATA	AAACTCTAAA	CAGACCTTGA	3780
35	CAAAATTAAT	CCTAGAGACT	GGCACAGACT	TACTTGGTAC	TCCTTCCCCT	TGCCCTATTT	3840
	AGAACTGAGA	ATACTCCCTC	TTGATTCCGT	TTTACTCTTT	TTAAGATCCT	TTATGGGGCT	3900
	CCTATGCCAT	CACTGTCTTA	AATGATGTGT	TTAAACCTAT	GTTGTTATAA	TAATGATCTA	3960
40	TATGTTAAGT	TAAAAGGCTT	GCAGGTGGTG	CAGAAAGAAG	TCTGGTCACA	ACTGGCTACA	4020
	GTGAACAAGC	TGGGTACCCC	AAGGACATCT	TACCAGTTCC	AGCCAGAGAT	CTGATCTACG	4080
45	TACACCTGCG	TCATGCTGAG	ACCCTCAAGC	CTCACTAAAA	GGGTCCCTGC	CTAGTTCTGT	4140
	TTACTAATCT	GCCTTATTCT	GTTTTTGTTT	CCATGTTAAA	GATAGAGTAA	ATGCAGTATT	4200
	CTCCACATAG	AGATATAGAC	TTCTGAAATT	CTAAGATTAG	AATTATTTAC	AAGAAGAAGT	4260
50	GGGGAATGAA	GAATAAAAAA	TTACTGGCCT	CTTGTGAGAA	CATGAACTTT	CACCTCGGAG	4320
	CCCACCCCTT	CCCATCTGGA	AAACATACTT	GAGAAAAACA	TTTTCTGGAA	CAACCACAGA	4380
55	ATGTTTCAAC	AGGCCAGATG	TATTGCCAAA	CACAGGATAT	GA CTCTTTGG	TTGAGTAAAT	4440
	TTGTGGTTGT	TAAACTTCCC	CTATTCCCTC	CCCATTCCCC	CTCCCAGTTT	GTGGTTTTTT	4500
	CCTTTAAAAG	CTTGTGAAAA	ATTTGAGTCG	TCGTCGAGAC	TCCTCTACCC	TGTGCAAAGG	4560
60	TGTATGAGTT	TCGACCCAG	AGCTCTGTGT	GCTTTCTGTT	GCTGCTTTAT	TTCGACCCCA	4620
	GAGCTCTGGT	CTGTGTGCTT	TCATGTCGCT	GCTTTATTAA	ATCTTACCTT	CTACATTTTA	4680
65	TGTATGGTCT	CAGTGTCTTC	TTGGGTACGC	GGCTGTCCCG	GGA CTTGAGT	GTCTGAGTGA	4740

GGGTCTTCCC TCGAGGGTCT TTCATTTGGT ACATGGGCCG GGAATTCGAG AATCTTTCAT 4800  
TTGGTGCATT GGCCGGGAAT TCGAAAATCT TTCA 4834

5 (2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 4518 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

20 CACCTGACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG 60  
TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTCTTTC CCTTCCTTTC 120  
TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC 180  
25 GATTTAGTGC TTTACGGCAC CTCGACCCCA AAAAATTGA TTAGGGTGAT GGTTCACGTA 240  
GTGGGCCATC GCCCTGATAG ACGTTTTTTC GCCCTTTGAC GTTGGAGTCC ACGTTCCTTA 300  
30 ATAGTGGACT CTTGTTCCAA ACTGGAACAA CACTCAACCC TATCTCGGTC TATTCTTTTG 360  
ATTTATAAGG GATTTTGCCG ATTTCGGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA 420  
AATTTAACGC GAATTTTAAC AAAATATTAA CGCTTACAAT TTACGCGTTA AGATACATTG 480  
35 ATGAGTTTGG ACAAAACCACA ACTAGAATGC AGTGAAAAAA ATGCTTTATT TGTGAAATTT 540  
GTGATGCTAT TGCTTTATTT GTAACCATTA TAAGCTGCAA TAAACAAGTT AACAACAACA 600  
40 ATTGCAATCA TTTTATGTTT CAGGTTTCAGG GGGAGGTGTG GGAGGTTTTT TAAAGCAAGT 660  
AAAACCTCTA CAAATGTGGT ATGGCTGATT ATGATCATGA ACAGACTGTG AGGACTGAGG 720  
GGCCTGAAAT GAGCCTTGGG ACTGTGAATC TAAAATACAC AAACAATTAG AATCAGTAGT 780  
45 TTAACACATT ATACACTTAA AAATTGGATC TCCATTGCGC ATTCAGGCTG CGCAACTGTT 840  
GGGAAGGGCG ATCGGTGCGG GCCTCTTCGC TATTACGCCA GCTGGCGAAA GGGGGATGTG 900  
50 CTGCAAGGCG ATTAAGTTGG GTAACGCCAG GGTTTTCCCA GTCACGACGT TGTA AACGA 960  
CGGCCAGTGA ATTGTAATAC GACTCACTAT AGGGCGAATT GGTACACTT ACCTGGTACC 1020  
CCACCCGGGT GGAAAATCGA TGGGCCCCGCG GCCGCTCTAG AAGTACTCTC GAGAAGCTTT 1080  
55 TTGAATTCTT TGGATCCACT AGTGTGACCG TGCAGGCGCG CGAGCTCCAG CTTTTGTTC 1140  
CTTTAGTGAG GGTAAATTTT GAGCTTGGCG TAATCAAGGT CATAGCTGTT TCCTGTGTGA 1200  
60 AATTGTTATC CGCTCACAAT TCCACACAAT ATACGAGCCG GAAGTATAAA GTGTAAAGCC 1260  
TGGGGTGCCT AATGAGTGAG CTAATCACA GTAAATGCGG CTAGCGGATC TGACGGTTCA 1320  
CTAAACCAGC TCTGCTTATA TAGACCTCCC ACCGTACACG CCTACCGCCC ATTTGCGTCA 1380  
65 ATGGGGCGGA GTTGTTACGA CATTTTGGAA AGTCCCGTTG ATTTTGGTGC CAAAACAAAC 1440

	TCCCATTGAC	GTCAATGGGG	TGGAGACTTG	GAAATCCCCG	TGAGTCAAAC	CGCTATCCAC	1500
5	GCCCATTGAT	GTAAGTCCAA	AACCGCATCA	CCATGGTAAT	AGCGATGACT	AATACGTAGA	1560
	TGTACTGCCA	AGTAGGAAAG	TCCCATAAGG	TCATGTACTG	GGCATAATGC	CAGGCGGGCC	1620
	ATTTACCGTC	ATTGACGTCA	ATAGGGGGCG	TACTTGGCAT	ATGATACT	TGATGTACTG	1680
10	CCAAGTGGGC	AGTTTACCGT	AAATACTCCA	CCCATTGACG	TCAATGGAAA	GTCCCTATTG	1740
	GCGTTACTAT	GGGAACATAC	GTCATTATTG	ACGTCAATGG	GCGGGGGTCG	TTGGGCGGTC	1800
15	AGCCAGGCGG	GCCATTTACC	GTAAGTTATG	TAACGCGGAA	CTCCATATAT	GGGCTATGAA	1860
	CTAATGACCC	CGTAATTGAT	TACTATTAAT	AACTAATGCA	TGGCGGTAAT	ACGGTTATCC	1920
	ACAGAATCAG	GGGATAACGC	AGGAAAGAAC	ATGTGAGCAA	AAGGCCAGCA	AAAGGCCAGG	1980
20	AACCGTAAAA	AGGCCGCGTT	GCTGGCGTTT	TTCCATAGGC	TCCGCCCCC	TGACGAGCAT	2040
	CACAAAAATC	GACGCTCAAG	TCAGAGGTGG	CGAAACCCGA	CAGGACTATA	AAGATACCAG	2100
	GCGTTTCCCC	CTGGAAGCTC	CCTCGTGCGC	TCTCCTGTTC	CGACCCTGCC	GCTTACCGGA	2160
25	TACCTGTCCG	CCTTTCTCCC	TTGCGGAAGC	GTGGCGCTTT	CTCATAGCTC	ACGCTGTAGG	2220
	TATCTCAGTT	CGGTGTAGGT	CGTTCGCTCC	AAGCTGGGCT	GTGTGCACGA	ACCCCCGTT	2280
30	CAGCCCGACC	GCTGCGCCTT	ATCCGGTAAC	TATCGTCTTG	AGTCCAACCC	GGTAAGACAC	2340
	GACTTATCGC	CACTGGCAGC	AGCCACTGGT	AACAGGATTA	GCAGAGCGAG	GTATGTAGGC	2400
	GGTGCTACAG	AGTTCTTGAA	GTGGTGGCCT	AACTACGGCT	ACACTAGAAG	GACAGTATTT	2460
35	GGTATCTGCG	CTCTGCTGAA	GCCAGTTACC	TTGCGAAAAA	GAGTTGGTAG	CTCTTGATCC	2520
	GGCAAACAAA	CCACCGCTGG	TAGCGGTGGT	TTTTTTGTTT	GCAAGCAGCA	GATTACGCGC	2580
40	AGAAAAAAG	GATCTCAAGA	AGATCCTTTG	ATCTTTTCTA	CGGGGTCTGA	CGCTCAGTGG	2640
	AACGAAACT	CACGTTAAGG	GATTTTGGTC	ATGAGATTAT	CAAAAAGGAT	CTTCACCTAG	2700
	ATCCTTTTAA	ATTAAAAATG	AAGTTTTTAA	TCAATCTAAA	GTATATATGA	GTAACCTGAG	2760
45	GCTATGGCAG	GGCCTGCCGC	CCCGACGTTG	GCTGCGAGCC	CTGGGCCTTC	ACCCGAACCT	2820
	GGGGGGTGGG	GTGGGGAAAA	GGAAGAAACG	CGGGCGTATT	GGCCCCAATG	GGGTCTCGGT	2880
50	GGGGTATCGA	CAGAGTGCCA	GCCCTGGGAC	CGAACCCCGC	GTTTATGAAC	AAACGACCCA	2940
	ACACCGTGCG	TTTTATTCTG	TCTTTTATT	GCCGTCATAG	CGCGGGTTCC	TTCCGGTATT	3000
	GTCTCCTTCC	GTGTTTTCAGT	TAGCCTCCCC	CTAGGGTGGG	CGAAGAACTC	CAGCATGAGA	3060
55	TCCCCGCGCT	GGAGGATCAT	CCAGCCGGCG	TCCCGAAAAA	CGATTCCGAA	GCCCAACCTT	3120
	TCATAGAAGG	CGGCGGTGGA	ATCGAAATCT	CGTGATGGCA	GGTTGGGCGT	CGCTTGGTGC	3180
60	GTCATTTTCA	ACCCAGAGT	CCCGCTCAGA	AGAACTCGTC	AAGAAGGCGA	TAGAAGGCGA	3240
	TGCGCTGCGA	ATCGGGAGCG	GCGATACCGT	AAAGCACGAG	GAAGCGGTCA	GCCCATTCGC	3300
	CGCCAAGCTC	TTCAGCAATA	TCACGGGTAG	CCAACGCTAT	GTCCTGATAG	CGGTCCGCCA	3360
65	CACCCAGCCG	GCCACAGTCG	ATGAATCCAG	AAAAGCGGCC	ATTTTCCACC	ATGATATTGC	3420

GCAAGCAGGC ATCGCCATGG GTCACGACGA GATCCTCGCC GTCGGGCATG CTCGCCTTGA 3480  
GCCTGGCGAA CAGTTCGGCT GGC GCGAGCC CCTGATGCTC TTCGTCCAGA TCATCCTGAT 3540  
5 CGACAAGACC GGCTTCCATC CGAGTACGTG CTCGCTCGAT GCGATGTTTC GCTTGGTGGT 3600  
CGAATGGGCA GGTAGCCGGA TCAAGCGTAT GCAGCCGCCG CATTGCATCA GCCATGATGG 3660  
10 ATACTTTCTC GGCAGGAGCA AGGTGAGATG ACAGGAGATC CTGCCCCGGC ACTTCGCCCA 3720  
ATAGCAGCCA GTCCCTTCCC GCTTCAGTGA CAACGTCGAG CACAGCTGCG CAAGGAACGC 3780  
CCGTCGTGGC CAGCCACGAT AGCCGCGCTG CCTCGTCTTG CAGTTCATTC AGGGCACC GG 3840  
15 ACAGGTCGGT CTTGACAAAA AGAACCGGGC GCCCCTGCGC TGACAGCCGG AACACGGCGG 3900  
CATCAGAGCA GCCGATTGTC TGTGTGCCC AGTCATAGCC GAATAGCCTC TCCACCCAAG 3960  
CGGCCGGAGA ACCTGCGTGC AATCCATCTT GTTCAATCAT GCGAAACGAT CCTCATCCTG 4020  
20 TCTCTTGATC GATCTTTGCA AAAGCCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG 4080  
AATAGCTCAG AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAT TAGTCAGCCA 4140  
TGGGGCGGAG AATGGGCGGA ACTGGGCGGA GTTAGGGGCG GGATGGGCGG AGTTAGGGGC 4200  
25 GGGACTATGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC TGCTGGGGAG 4260  
CCTGGGGACT TTCCACACCT GGTGCTGAC TAATTGAGAT GCATGCTTTG CATACTTCTG 4320  
30 CCTGCTGGGG AGCCTGGGGA CTTTCCACAC CTAAGTAC ACACATTCCA CAGCTGGTTC 4380  
TTTCCGCCTC AGGACTCTTC CTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC 4440  
TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA 4500  
35 CATTTCCCCG AAAAGTGC 4518

## (2) INFORMATION FOR SEQ ID NO:27:

- 40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
45 (ii) MOLECULE TYPE: DNA (genomic)

- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTCCACATAG AGATATAGAC TTCTG

25

- 55 (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
60 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)

65



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGATCTTATT AATTAAGTGG AGTTTGTGAGC CCRMCCCCCTC CCATC

45

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5594 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

20	TGCATTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG TTCATAGCCC ATATATGGAG	60
	TTCCGCGTTA CATAACTTAC GGTAATGGC CCGCTGGCT GACCGCCCAA CGACCCCCGC	120
25	CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC CAATAGGGAC TTTCCATTGA	180
	CGTCAATGGG TGGAGTATTT ACGGTAACT GCCCACTTGG CAGTACATCA AGTGTATCAT	240
	ATGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT GGCCCGCCTG GCATTATGCC	300
30	CAGTACATGA CCTTATGGGA CTTTCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT	360
	ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA	420
35	CGGGGATTTT CAAGTCTCCA CCCCATGAC GTCAATGGGA GTTTGTTTTG GCACCAAAAT	480
	CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCAT TGACGCAAAT GGGCGGTAGG	540
	CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTGGTTTAG TGAACCGTCA GATCCGCGCC	600
40	AGTCCTCCGA TTGACTGAGT CGCCCGGGTA CCCGTGTATC CAATAAACCC TCTTGCAGTT	660
	GCATCCGACT TGTGGTCTCG CTGTTCTTGG GGAGGGTCTC CTCTGAGTGA TTGACTACCC	720
45	GTCAGCGGGG GTCTTTCATT TGGGGGCTCG TCCGGGATCG GGAGACCCCT GCCCAGGGAC	780
	CACCGACCCA CCACCGGGAG GTAAGCTGGC CAGCAACTTA TCTGTGTCTG TCCGATTGTC	840
	TAGTGTCTAT GACTGATTTT ATGCGCCTGC GTCGGTACTA GTTAGCTAAC TAGCTCTGTA	900
50	TCTGGCGGAC CCGTGGTGGG ACTGACGAGT TCGGAACACC CGGCCGCAAC CCTGGGAGAC	960
	GTCCAGGAG GAACAGGGGA GGATCAGGGA CGCCTGGTGG ACCCCTTTGA AGGCAAGAG	1020
55	ACCATTGTTGG GTTGCGAGAT CGTGGGTTCG AGTCCACCT CGTGCCAGT TGCAGATCG	1080
	TGGGTTCGAG TCCACCTCG TGTGTTGTTG CGAGATCGTG GGTTCGAGTC CCACCTCGCG	1140
	TCTGGTCACG GGATCGTGGG TTCGAGTCCC ACCTCGTGTT TTGTTGCGAG ATCGTGGGTT	1200
60	CGAGTCCCAC CTCGCTCTG GTCACGGGAT CGTGGGTTCG AGTCCACCT CGTGCAGAGG	1260
	GTCTCAATTG GCCGGCCTTA GAGAGGCCAT CTGATTCTTC TGGTTTCTCT TTTTGTCTTA	1320
65	GTCTCGTGTC CGCTCTTGTT GTGACTACTG TTTTCTAAA AATGGGACAA TCTGTGTCCA	1380
	CTCCCTTTT TCTGACTCTG GTTCTGTCGC TTGGTAATTT TGTTTGTTTA CGTTTGTTTT	1440

	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	TTGTTTTTGT	TTGTGGTTTA	CGGTTTCTGT	1500
	GTGTGTCTTG	TGTGTCTCTT	TGTGTTTCTT	CTTGGACTGA	TGACTGACGA	CTGTTTTTAA	1560
5	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	1620
	TCAGATCAAC	AGCTGCCCTT	ACGTATCGAT	GGATCCCTCG	ACTAACTAAT	AGCCCATTCT	1680
10	CCAAGGTCGA	GCGGGATCAA	TTCCGCCCCC	CCCCTAACGT	TACTGGCCGA	AGCCGCTTGG	1740
	AATAAGGCCG	GTGTGCGTTT	GTCTATATGT	TATTTTCCAC	CATATTGCCG	TCTTTTGGCA	1800
	ATGTGAGGGC	CCGGAAACCT	GGCCCTGTCT	TCTTGACGAG	CATTCTTAGG	GGTCTTTCCC	1860
15	CTCTCGCCAA	AGGAATGCAA	GGTCTGTTGA	ATGTCGTGAA	GGAAGCAGTT	CCTCTGGAAG	1920
	CTTCTTGAAG	ACAAACAACG	TCTGTAGCGA	CCCTTTGCAG	GCAGCGGAAC	CCCCACCTG	1980
20	GCGACAGGTG	CCTCTGCGGC	CAAAAGCCAC	GTGTATAAGA	TACACCTGCA	AAGGCGGCAC	2040
	AACCCAGTG	CCACGTTGTG	AGTTGGATAG	TTGTGGAAAG	AGTCAAATGG	CTCTCCTCAA	2100
	GCGTATTCAA	CAAGGGGCTG	AAGGATGCCC	AGAAGGTACC	CCATTGTATG	GGATCTGATC	2160
25	TGGGGCCTCG	GTGCACATGC	TTTACATGTG	TTTAGTCGAG	GTTAAAAAAA	CGTCTAGGCC	2220
	CCCCGAACCA	CGGGGACGTG	GTTTTTCCTT	GAAAAACACG	ATAATAATCA	TGGCTACAGG	2280
30	CTCCCGGACG	TCCCTGCTCC	TGGCTTTTGG	CCTGCTCTGC	CTGCCCTGGC	TTCAAGAGGG	2340
	CAGTGCCCTC	CCAACCATTC	CCTTATCCAG	GCTTTTTGAC	AACGCTATGC	TCCGCGCCCA	2400
	TCGTCTGCAC	CAGCTGGCCT	TTGACACCTA	CCAGGAGTTT	GAAGAAGCCT	ATATCCCCAA	2460
35	GGAACAGAAG	TATTCATTCC	TGCAGAACCC	CCAGACCTCC	CTCTGTTTCT	CAGAGTCTAT	2520
	TCCGACACCC	TCCAACAGGG	AGGAAACACA	ACAGAAATCC	AACCTAGAGC	TGCTCCGCAT	2580
40	CTCCCTGCTG	CTCATCCAGT	CGTGGCTGGA	GCCCGTGCAG	TTCTCAGGA	GTGTCTTCGC	2640
	CAACAGCCTG	GTGTACGGCG	CCTCTGACAG	CAACGTCTAT	GACCTCCTAA	AGGACCTAGA	2700
	GGAAGGCATC	CAAACGCTGA	TGGGGAGGCT	GGAAGATGGC	AGCCCCCGGA	CTGGGCAGAT	2760
45	CTTCAAGCAG	ACCTACAGCA	AGTTGACAC	AAACTCACAC	AACGATGACG	CACTACTCAA	2820
	GAACTACGGG	CTGCTCTACT	GCTTCAGGAA	GGACATGGAC	AAGGTCGAGA	CATTCTTGCG	2880
50	CATCGTGCAG	TGCCGCTCTG	TGGAGGGCAG	CTGTGGCTTC	TAGCTGCCCC	GGTGGCATCC	2940
	TGTGACCCCT	CCCCAGTGCC	TCTCCTGGCC	CTGGAAGTTG	CCACTCCAGT	GCCCACCAGC	3000
	CTTGTCCTAA	TGTGTGTCAG	TTAGGGTGTG	GAAAGTCCCC	AGGCTCCCCA	GCAGGCAGAA	3060
55	GTATGCAAAG	CATGCATCTC	AATTAGTCAG	CAACCAGGTG	TGGAAAGTCC	CCAGGCTCCC	3120
	CAGCAGGCAG	AAGTATGCAA	AGCATGCATC	TCAATTAGTC	AGCAACCATA	GTCCCGCCCC	3180
60	TAACCTCCGCC	CATCCCGCCC	CTAACTCCGC	CCAGTTCCGC	CCATTCTCCG	CCCCATGGCT	3240
	GACTAATTTT	TTTTATTTAT	GCAGAGGCCG	AGGCCGCCCT	GGCCTCTGAG	CTATTCCAGA	3300
	AGTAGTGAGG	AGGCTTTTTT	GGAGGCCTAG	GCTTTTGCAA	AAAGCTTCAC	GCTGCCGCAA	3360
65	GCACTCAGGG	CGCAAGGGCT	GCTAAAGGAA	GCGGAACACG	TAGAAAGCCA	GTCCGCAGAA	3420

	ACGGTGCTGA	CCCCGGATGA	ATGTCAGCTA	CTGGGCTATC	TGGACAAGGG	AAAACGCAAG	3480
5	CGCAAAGAGA	AAGCAGGTAG	CTTGCCAGTGG	GCTTACATGG	CGATAGCTAG	ACTGGGCGGT	3540
	TTTATGGACA	GCAAGCGAAC	CGGAATTGCC	AGCTGGGGCG	CCCTCTGGTA	AGGTTGGGAA	3600
	GCCCTGCAAA	GTAAACTGGA	TGGCTTTCTT	GCCGCCAAGG	ATCTGATGGC	GCAGGGGATC	3660
10	AAGATCTGAT	CAAGAGACAG	GATGAGGATC	GTTTCGCATG	ATTGAACAAG	ATGGATTGCA	3720
	CGCAGGTTCT	CCGGCCGCTT	GGGTGGAGAG	GCTATTCGGC	TATGACTGGG	CACAACAGAC	3780
	AATCGGCTGC	TCTGATGCCG	CCGTGTTCCG	GCTGTCAGCG	CAGGGGCGCC	CGGTTCTTTT	3840
15	TGTCAAGACC	GACCTGTCCG	GTGCCCTGAA	TGAACTGCAG	GACGAGGCAG	CGCGGCTATC	3900
	GTGGCTGGCC	ACGACGGGCG	TTCCTTGCGC	AGCTGTGCTC	GACGTTGTCA	CTGAAGCGGG	3960
20	AAGGGACTGG	CTGCTATTGG	GCGAAGTGCC	GGGGCAGGAT	CTCCTGTCAT	CTCACCTTGC	4020
	TCCTGCCGAG	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG	CGGCTGCATA	CGCTTGATCC	4080
	GGCTACCTGC	CCATTGACC	ACCAAGCGAA	ACATCGCATC	GAGCGAGCAC	GTACTCGGAT	4140
25	GGAAGCCGGT	CTTGTCGATC	AGGATGATCT	GGACGAAGAG	CATCAGGGGC	TCGCGCCAGC	4200
	CGAACTGTTC	GCCAGGCTCA	AGGCGCGCAT	GCCCGACGGC	GAGGATCTCG	TCGTGACCCA	4260
30	TGGCGATGCC	TGCTTGCCGA	ATATCATGGT	GGAAAATGGC	CGCTTTTCTG	GATTCATCGA	4320
	CTGTGGCCGG	CTGGGTGTGG	CGGACCGCTA	TCAGGACATA	GCGTTGGCTA	CCCGTGATAT	4380
	TGCTGAAGAG	CTTGCGGGCG	AATGGGCTGA	CCGCTTCCTC	GTGCTTTACG	GTATCGCCGC	4440
35	TCCCGATTCTG	CAGCGCATCG	CCTTCTATCG	CCTTCTTGAC	GAGTTCTTCT	GAGCGGGACT	4500
	CTGGGGTTCTG	AAATGACCGA	CCAAGCGACG	CCCAACCTCC	AGAAAAAGGG	GGGAATGAAA	4560
40	GACCCACCT	GTAGGTTTGG	CAAGCTAGCT	TAAGTAACGC	CATTTTGCAA	GGCATGGAAA	4620
	AATACATAAC	TGAGAATAGA	GAAGTTCAGA	TCAAGGTCAG	GAACAGATGG	AACAGCTGAA	4680
	TATGGGCCAA	ACAGGATATC	TGTGGTAAGC	AGTTCCTGCC	CCGGCTCAGG	GCCAAGAACA	4740
45	GATGGAACAG	CTGAATATGG	GCCAAACAGG	ATATCTGTGG	TAAGCAGTTC	CTGCCCCGGC	4800
	TCAGGGCCAA	GAACAGATGG	TCCCCAGATG	CGGTCCAGCC	CTCAGCAGTT	TCTAGAGAAC	4860
50	CATCAGATGT	TTCCAGGGTG	CCCCAAGGAC	CTGAAATGAC	CCTGTGCCTT	ATTTGAACTA	4920
	ACCAATCAGT	TCGCTTCTCG	CTTCTGTTCG	CGCGCTTCTG	CTCCCCGAGC	TCAATAAAAG	4980
	AGCCCACAAC	CCCTCACTCG	GGGCGCCAGT	AATCTGCTGC	TTGCAAACAA	AAAAACCACC	5040
55	GCTACCAGCG	GTGGTTTGT	TGCCGGATCA	AGAGCTACCA	ACTCTTTTTC	CGAAGGTAAC	5100
	TGGCTTCAGC	AGAGCGCAGA	TACCAAATAC	TGTCCTTCTA	GTGTAGCCGT	AGTTAGGCCA	5160
60	CCACTTCAAG	AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	5220
	GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	GACTCAAGAC	GATAGTTACC	5280
	GGATAAGGCG	CAGCGGTCGG	GCTGAACGGG	GGGTTCGTGC	ACACAGCCCA	GCTTGGAGCG	5340
65	AACGACCTAC	ACCGAACTGA	GATACCTACA	GCGTGAGCAT	TGAGAAAGCG	CCACGCTTCC	5400

CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC 5460  
GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT 5520  
5 CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC 5580  
CAGCAACGCC GAGA 5594

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6561 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GATCCCCGGG TCGACCCGGG TCGACCCTGT GGAATGTGTG TCAGTTAGGG TGTGGAAAGT 60  
25 CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA TCTCAATTAG TCAGCAACCA 120  
GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT 180  
30 AGTCAGCAAC CATAGTCCCG CCCCTAACTC CGCCCATCCC GCCCCTAACT CCGCCCAGTT 240  
CCGCCCATT CCGCCCCAT GGCTGACTAA TTTTTTTTAT TTATGCAGAG GCCGAGGCCG 300  
CCTCGGCCTC TGAGCTATTC CAGAAGTAGT GAGGAGGCTT TTTTGGAGGC CTAGGCTTTT 360  
35 GCAAAAAGCT TCACGCTGCC GCAAGCACTC AGGGCGCAAG GGCTGCTAAA GGAAGCGGAA 420  
CACGTAGAAA GCCAGTCCGC AGAAACGGTG CTGACCCCGG ATGAATGTCA GCTACTGGGC 480  
40 TATCTGGACA AGGGAAAACG CAAGCGCAAA GAGAAAGCAG GTAGCTTGCA GTGGGCTTAC 540  
ATGGCGATAG CTAGACTGGG CGGTTTTATG GACAGCAAGC GAACCGGAAT TGCCAGCTGG 600  
GGCGCCCTCT GGTAAGGTTG GGAAGCCCTG CAAAGTAAAC TGGATGGCTT TCTTGCCGCC 660  
45 AAGGATCTGA TGGCGCAGGG GATCAAGATC TGATCAAGAG ACAGGATGAG GATCGTTTCG 720  
CATGATTGAA CAAGATGGAT TGCACGCAGG TTCTCCGGCC GCTTGGGTGG AGAGGCTATT 780  
50 CGGCTATGAC TGGGCACAAC AGACAATCGG CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC 840  
AGCGCAGGGG CGCCCGGTTT TTTTGTCAA GACCGACCTG TCCGGTGCCC TGAATGAACT 900  
GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GGCGTTCCTT GCGCAGCTGT 960  
55 GCTCGACGTT GTCAGTGAAG CGGGAAGGGA CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA 1020  
GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT 1080  
60 GCGGCGGGCTG CATACGCTTG ATCCGGCTAC CTGCCCATTC GACCACCAAG CGAAACATCG 1140  
CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA 1200  
AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG CTCAAGGCGC GCATGCCCGA 1260  
65 CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG CCGAATATCA TGGTGGAAAA 1320

	TGGCCGCTTT	TCTGGATTCA	TCGACTGTGG	CCGGCTGGGT	GTGGCGGACC	GCTATCAGGA	1380
	CATAGCGTTG	GCTACCCGTG	ATATTGCTGA	AGAGCTTGGC	GGCGAATGGG	CTGACCGCTT	1440
5	CCTCGTGCTT	TACGGTATCG	CCGCTCCCGA	TTCGCAGCGC	ATCGCCTTCT	ATCGCCTTCT	1500
	TGACGAGTTC	TTCTGAGCGG	GACTCTGGGG	TTCGAAATGA	CCGACCAAGC	GACGCCCAAC	1560
10	CTGCCATCAC	GAGATTTTCA	TTCCACCGCC	GCCTTCTATG	AAAGGTTGGG	CTTCGGAATC	1620
	GTTTTCCGGG	ACGCCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT	GGAGTTCTTC	1680
	GCCCCACCCG	GAATTCGTAA	TCTGCTGCTT	GCAAACAAAA	AAACCACCGC	TACCAGCGGT	1740
15	GGTTTGTGTT	CCGGATCAAG	AGCTACCAAC	TCTTTTTTCCG	AAGGTAAGTG	GCTTCAGCAG	1800
	AGCGCAGATA	CCAAATACTG	TCCTTCTAGT	GTAGCCGTAG	TTAGGCCACC	ACTTCAAGAA	1860
20	CTCTGTAGCA	CCGCCTACAT	ACCTCGCTCT	GCTAATCCTG	TTACCAGTGG	CTGCTGCCAG	1920
	TGGCGATAAG	TCGTGTCTTA	CCGGGTTGGA	CTCAAGACGA	TAGTTACCGG	ATAAGGCGCA	1980
	GCGGTCGGGC	TGAACGGGGG	GTTCGTGCAC	ACAGCCCAGC	TTGGAGCGAA	CGACCTACAC	2040
25	CGAACTGAGA	TACCTACAGC	GTGAGCATTG	AGAAAGCGCC	ACGCTTCCCG	AAGGGAGAAA	2100
	GGCGGACAGG	TATCCGGTAA	GCGGCAGGGT	CGGAACAGGA	GAGCGCACGA	GGGAGCTTCC	2160
30	AGGGGGAAAC	GCCTGGTATC	TTTATAGTCC	TGTCGGGTTT	CGCCACCTCT	GACTTGAGCG	2220
	TCGATTTTTG	TGATGCTCGT	CAGGGGGGCG	GAGCCTATGG	AAAAACGCCA	GCAACGCCGA	2280
	GATGCGCCGC	CTCGAGTACA	CCTGCGTCAT	GCTGAGACCC	TCAAGCCTCA	CTAAAAGGGT	2340
35	CCCTGCCTAG	TTCTGTTTAC	TAATCTGCCT	TATTCTGTTT	TTGTTCCCAT	GTTAAAGATA	2400
	GAGTAAATGC	AGTATTCTCC	ACATAGAGAT	ATAGACTTCT	GAAATTCTAA	GATTAGAATT	2460
40	ATTTACAAGA	AGAAGTGGGG	AATGAAGAAT	AAAAAATTAC	TGGCCTCTTG	TGAGAACATG	2520
	AACTTTCACC	TCGGAGCCCA	CCCCCTCCCA	TCTGGAAAAC	ATACTTGAGA	AAAACATTTT	2580
	CTGGAACAAC	CACAGAATGT	TTCAACAGGC	CAGATGTATT	GCCAAACACA	GGATATGACT	2640
45	CTTTGGTTGA	GTAAATTTGT	GGTTGTTAAA	CTTCCCCTAT	TCCCTCCCCA	TTCCCCCTCC	2700
	CAGTTTGTGG	TTTTTTCCTT	TAAAAGCTTG	TGAAAAATTT	GAGTCGTCGT	CGAGACTCCT	2760
50	CTACCCTGTG	CAAAGGTGTA	TGAGTTTCGA	CCCCAGAGCT	CTGTGTGCTT	TCTGTTGCTG	2820
	CTTTATTTTCG	ACCCAGAGC	TCTGGTCTGT	GTGCTTTCAT	GTCGCTGCTT	TATTAAATCT	2880
	TACCTTCTAC	ATTTTATGTA	TGGTCTCAGT	GTCTTCTTGG	GTACGCGGCT	GTCCCGGGAC	2940
55	TTGAGTGTCT	GAGTGAGGGT	CTTCCCTCGA	GGGTCTTTCA	TTTGGTACAT	GGGCCGGGAA	3000
	TTGAGAATC	TTTCATTTGG	TGCATTGGCC	GGGAATTCGA	AAATCTTTCA	TTTGGTGCAT	3060
60	TGGCCGGGAA	ACAGCGCGAC	CACCCAGAGG	TCCTAGACCC	ACTTAGAGGT	AAGATTCTTT	3120
	GTTCTGTTTT	GGTCTGATGT	CTGTGTTCTG	ATGTCTGTGT	TCTGTTTCTA	AGTCTGGTGC	3180
	GATCGCAGTT	TCAGTTTTGC	GGACGCTCAG	TGAGACCGCG	CTCCGAGAGG	GAGTGCGGGG	3240
65	TGGATAAGGA	TAGACGTGTC	CAGGTGTCCA	CCGTCCGTTC	GCCCTGGGAG	ACGTCCCAGG	3300

	AGGAACAGGG	GAGGATCAGG	GACGCCTGGT	GGACCCCTTT	GAAGGCCAAG	AGACCATTTG	3360
	GGGTTGCGAG	ATCGTGGGTT	CGAGTCCCAC	CTCGTGCCCA	GTTGCGAGAT	CGTGGGTTTCG	3420
5	AGTCCCACCT	CGTGTTTTGT	TGCGAGATCG	TGGGTTTCGAG	TCCCACCTCG	CGTCTGGTCA	3480
	CGGGATCGTG	GGTTCGAGTC	CCACCTCGTG	TTTTGTTGCG	AGATCGTGGG	TTGAGTCCC	3540
10	ACCTCGCGTC	TGGTCACGGG	ATCGTGGGTT	CGAGTCCCAC	CTCGTGCAGA	GGGTCTCAAT	3600
	TGGCCGGCCT	TAGAGAGGCC	ATCTGATTCT	TCTGGTTTCT	CTTTTGTCT	TAGTCTCGTG	3660
	TCCGCTCTTG	TTGTGACTAC	TGTTTTTCTA	AAAATGGGAC	AATCTGTGTC	CACTCCCCTT	3720
15	TCTCTGACTC	TGGTTCTGTC	GCTTGGAAT	TTTGTGTTGTT	TACGTTTGTT	TTTGTGAGTC	3780
	GTCTATGTTG	TCTGTTACTA	TCTTGTTTTT	GTTTGTGGTT	TACGGTTTCT	GTGTGTGTCT	3840
	TGTGTGTCTC	TTTGTGTTCA	GACTIONGACT	GATGACTGAC	GACTIONTTTT	AAGTTATGCC	3900
20	TTCTAAAATA	AGCCTAAAAA	TCCTGTCAGA	TCCCTATGCT	GACCACTTCC	TTTCAGATCA	3960
	ACAGCTGCCC	TGCCTCCCAC	TCCAACCTCA	GAGAGCAGCC	AGCGGGTCAC	AGTGGTCCCCG	4020
25	CCCATGAACC	TGGAGCCTAG	GGAAAAATGA	GCTCGGAAAT	CCGGAGCAAA	TGAGGAGTGG	4080
	TCCCTGAGAA	GTCAGTGGCC	TAAATGTTGT	GGCTGCTGAA	GCAAAAGAAG	AGGAGGCTGT	4140
	TCGAGTAGCC	GGCCAAGAGC	GCCGCGGGTT	CCCAGGCAGC	TTCTCATTC	CCTGTCCCTC	4200
30	CCATCCCGTC	TCTTGTTAAC	AGAAAACTG	CTTTCACCTT	GAGATATGAG	TGGCCCGATA	4260
	CAGCCAGCTG	TGAGAGCTGT	ACTCCCTTCC	CTGCCCCACG	TGTTTTCTCT	TCTCAGGCGA	4320
35	CCCCTCCCTG	AGCTGCTGGC	AGTGAGTCTG	TTCTAAGCTC	CAGTGAGGGA	GGCATCCGCC	4380
	CACTTGGGGC	TTCTGTCCAA	GGTAAGGAGC	ACCTGTGAGT	CTAACTGCCA	GGCTCTGATG	4440
	GGGGTCTCGT	CTCTGTGGGA	CTAGAAAGTG	TCCCAACAAT	CTGACCAAGG	TAACAGGAAG	4500
40	TTAAGACAAA	GACAGAGACC	AAAGTCAGAA	TCAGAGCTGT	GCTGTGAGAC	AAAAAGATAA	4560
	AAAAAATAAA	ATGCTGGCCA	CAAAAGTCAG	GAAAACTAGA	AAACTTAGAT	AGTACCTGGC	4620
45	AACAAAAGAA	AGCTTTTGGC	TAAAGATCAA	CGTGTATACT	GTAAAGAAAA	TGAGCACTGG	4680
	GTGAGAGACT	GCCCCAACAA	AAAGAAGAGG	AGCCCCCTC	ATGACCAAAC	CCTTCACCTG	4740
	TTCGTGGCTA	AAAGTAAAGA	GATAACAAAA	GGGGTGCTAA	CACAGAAGCT	GAGTCCTTAA	4800
50	AAGAGTCCGG	TGGCCTACCT	GTTGAAGCAG	CTAAAAAAGA	GACTGTGTTT	CATACTCCTC	4860
	CACTGACCAG	TGCAAAACAA	GCTAAAAAGT	TCCTGGGCAC	TGCGGGCTTT	TGCAGATTGT	4920
55	GGATTCCAGG	TTTTGCTGAG	TTAAAGAGAT	AAACAGCCCT	TCGTATAGAA	AAATAAAAAA	4980
	CAACCTTGGA	TGTCCTTGGA	TGCTATTGAG	ACTGCCCTAA	TGTTGTCCCC	AGCTATGGGA	5040
	CTCCTAGATG	TGACTGAGAA	CAAAGGTATT	GCCAAAGAAG	TTCTTACTCA	GAGATTGGGA	5100
60	CCCTGAAAAA	GACCTGTGGC	ATACTTGTA	GAAATTAGAC	CTGGTGGCTG	TAAGATGGCC	5160
	TGCTTGTCTG	CACATAGTGG	CTTCTGGTCA	AGGACGCAGA	TAAATTGACT	CTGAGACAAA	5220
	ACTTGGCACA	TGTCCTAGAA	AGTGTGGTTC	AGCCCCCATG	ACCGATGGCT	GACTIONACGCT	5280
65	CTTGAAAAACA	TTATCCAAC	GTTCCCCCTGA	CCGATGGACA	CATTGTCAGA	GCTTTTTTTT	5340

ACTGAACGAG TGACCTTCGC TCCCCCTGCT ATCCTCGATC TCACTACTGC CTGAGACTTC 5400  
ACCTACTCAT CATTGTGCTG ACATTCTGGC AGAAGAAACT CATACTCGAA ATGATCTGAA 5460  
5 GGATCAGATC AGCCTTGGCC TGAGAGTTTG AGCTGGTACA CGGATGGCAG TAGCCTGGAG 5520  
GTTAAGGGTA AGCGGAAGGC GGGGACAGCA GTGCAGTGGT GGACAGAAAG CAAGTGATCT 5580  
10 AGGCCAGCAG CCTCCCTAAA GGGACTTCAG CCCACAAAGC CAAACTTGTG GCTTTAATAC 5640  
AAGCTCTGTA AATGGTAAAA AAAAAAAGT CTACACGGAC AGCAGGTATG CTCTTGCCAC 5700  
TGTACAGAGC AATATACAGA CAAAGAGAAC TGTTGACATC TGCAGAGAAA GACCTAAGAT 5760  
15 GCTGTGGCTA AAAGAAATCA GATGGCAAAT CTAACCGCCC AGGCATCCTA AAGAGCAATG 5820  
ATCCTGACAG TCTGAAGACT ATCAAGTTAT AGACAAATTA AGACTGGTAA AAAAAACCTT 5880  
GTATAAAATA GTAAAAACTG AAAAAAGAAA ACTAGTCCTC TCATGAGAAG ACAGACCTGA 5940  
20 CATCTACTGA AAAATAGACT TTACTGGAAA AAATATGTGT ATGAATACCT TCTAGTTTTT 6000  
GTGAACGTTT TCAAGATGGA TAAAAGCTTT TCCTTGTAAG ACGAGACTGA TCAGATAGTC 6060  
25 ATCAAGAAGA TTGTTAAAGA AAATTTTCCA AGGTTCCGGAG TGCCAAAAGC AATAGTGTC 6120  
GATAATGGTC CTGCCTTTGT TGCCCAGGTA AGTCAGGGTG TGGCCAAGTA TTTAGAGGTC 6180  
AAATGAAAAT TCCATTGTGT GTACAGACCT CAGAGCTCAG GAAAGATAAA AAAGAATAAA 6240  
30 TAAACTCTA AACAGACCTT GACAAAATTA ATCCTAGAGA CTGGCACAGA CTTACTTGGT 6300  
ACTCCTTCCC CTTGCCCTAT TTAGAACTGA GAATACTCCC TCTTGATTCTG GTTTTACTCT 6360  
35 TTTTAAGATC CTTTATGGGG CTCCTATGCC ATCACTGTCT TAAATGATGT GTTTAAACCT 6420  
ATGTTGTTAT AATAATGATC TATATGTTAA GTTAAAAGGC TTGCAGGTGG TGCAGAAAGA 6480  
AGTCTGGTCA CAACTGGCTA CAGTGAACAA GCTGGGTACC CCAAGGACAT CTTACCAGTT 6540  
40 CCAGCCAGAG ATCTGATCTA C 6561

(2) INFORMATION FOR SEQ ID NO:31:

45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 55 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
50 (ii) MOLECULE TYPE: DNA (genomic)

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GACTAACCTT GATTCCACTG GAGCCGTATT ACCGCCATGC ATTAGTTATT AATAG 55

60 (2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 47 base pairs  
(B) TYPE: nucleic acid  
65 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GACTAACCTT GATTCCACTG GAGTAATTGC GGCTAGCGGA TCTGACG

47

10 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

25 GACTAACCTT GATTCCACTG GAGACACTTG ACCTCTACCG CGCCAGTCCT CCGATTGACT

60

GAGTCG

66

(2) INFORMATION FOR SEQ ID NO:34:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs

(B) TYPE: nucleic acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

45 GACTAACCTT GATTCCACTG GAGGGATCCG CGCCCATGAT TATTATCG

48

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 55 base pairs

50 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

60

GACTAACCTT GATTCCAGCA ATGTCATGGC TACAGGCTCC CGGACGTCCC TGCTC

55

(2) INFORMATION FOR SEQ ID NO:36:

65

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs



(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

10 GACTAACCTT GATTCCAGCA ATGTTAGGAC AAGGCTGGTG GGCCTGG 48

(2) INFORMATION FOR SEQ ID NO:37:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 49 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACTAACCTT GATTCCACTG GAGGGTCGAC CCTGTGGAAT GTGTGTCAG 49

30 (2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 48 base pairs  
(B) TYPE: nucleic acid  
35 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

45 GACTAACCTT GATTCCACTG GAGAATCTCG TGATGGCAGG TTGGGCGT 48

(2) INFORMATION FOR SEQ ID NO:39:

50 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 54 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GACTAACCTT GATTCCACTG AAGAGATTTT ATTTAGTCTC CAGAAAAAGG GGGG 54

(2) INFORMATION FOR SEQ ID NO:40:

65 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GACTAACCTT GATTCCACTG AAGCCCCCAA ATGAAAGACC CCCGCTGACG

50

15

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: DNA (genomic)

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

30

GACTAACCTT GATTCCACTG GAGCCGGGAC GGAATTCGTA ATCTGCTGC

49

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

40

(ii) MOLECULE TYPE: DNA (genomic)

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GACTAACCTT GATTCCACTG GAGTTCTCGA GCGGGCGCAT CTCGGCG

47

(2) INFORMATION FOR SEQ ID NO:43:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: DNA (genomic)

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CGCTCTAGAA CTAGTGGATC

20

65

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:  
5 (A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

15 GTAATACGAC TCACTATAGG G 21

(2) INFORMATION FOR SEQ ID NO:45:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CGATCCACTG GAGCTCGGAG CCCACCCCCT CCCATCTAGA GGT 43

(2) INFORMATION FOR SEQ ID NO:46:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

50 CGTCCTCCTG GAGAGCACAG GGTAGAGGAG TCTCGACGGT CAG 43

(2) INFORMATION FOR SEQ ID NO:47:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

65 CGCAACCCTG GAGACCTCTA GATGGGAGGG GGTGGGCTCC GAG 43

## (2) INFORMATION FOR SEQ ID NO:48:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCAGGACCTG GAGCTGACCG TCGAGACTCC TCTACCCTGT GCT

43

## (2) INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGCTCTAGAA CTAGTGGATC

20

## (2) INFORMATION FOR SEQ ID NO:50:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GTAATACGAC TCACTATAGG G

21

## (2) INFORMATION FOR SEQ ID NO:51:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TACGTATCGA TGGATCCGA

19

(2) INFORMATION FOR SEQ ID NO:52:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: DNA (genomic)

- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GGATCCATCG ATACGTAAG

19

20 (2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 38 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: DNA (genomic)
- 30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

35 GGCCGCTAAC TAATAGCCCA TTCTCCAAGG TACGTAGC

38

(2) INFORMATION FOR SEQ ID NO:54:

- 40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 38 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: DNA (genomic)

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TACGTACCTT GGAGAATGGG CTATTAGTTA GCGGCCGC

38

(2) INFORMATION FOR SEQ ID NO:55:

- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 55 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: DNA (genomic)
- 65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:  
GACTAACCTT GATTCCACTG GAGTTTTCTC TATTCTTCAT TCCCCACTTC TTCTT 55

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GACTAACCTT GATTCCACTG GAGAATCTGG ACCAATTCTA TATAAGCCTG TGAAAAATTT 60

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GACTAACCTT GATTCCACTG GAGAAGAAGA AGTGGGGAAT GAAGAA 46

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GACTAACCTT GATTCCACTG GAGATCTCTA GATGGGAGGG GGTCTGGGCT C 51

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GACTAACCTT GATTCCACTG GAGCTCGGAG CCCACCCCCT CCCATCT

47

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GACTAACCTT GATTCCACTG GAGGGAGGCC CTTATCTCAA AAATGTT

47

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GACTAACCTT GATTCCACTG GAGTCTAAGA ACATTTTGA GATAAGGGCC T

51

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GACTAACCTT GATTCCACTG GAGTCACAGG CTTATATAGT GAAA

44

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

5 GACTAACCTT GATTCCCTGG AGACTGCACT GCTGTCCCCG CCTTCG 46

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 53 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

20 GAGTAACCTT GATTCCCTGG AGATTTCTCA GACCCGGGTC GACCCTGTGG AAT 53

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 44 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

35 GACTAACCTT GATTCCCTGG AGCTCGAGGC GGCGCATCTC GGCG 44

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 47 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

50 GACTAACCTT GATTCCCTGA AGACCTGCGT CATGCTGAGA CCCTCAA 47

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

60 (A) LENGTH: 50 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

65 (ii) MOLECULE TYPE: DNA (genomic)



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

5

GACTAACCTT GATTCCCTGA AGCGGCCAAT GCACCAAATG AAAGATTTTC

50

(2) INFORMATION FOR SEQ ID NO:68:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CGCATCTTTT AATTAAGTGG AGAATTTT TYACAGGCTT ATATAGKAAA

50

We claim:

1. A method for assembling a gene or gene vector comprising the steps of:

5                   a) designing at least 6 primers to produce at least three fragments in at least three separate polymerase chain reactions wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template sequence for amplification, and bases positioned at the restriction endonuclease  
10                   cleavage site that are selected to be complementary to only one other overhanging created from enzymatic cleavage of the fragments;

                  b) combining the primers with template nucleic acid and performing a gene amplification reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site;

15                   c) digesting the amplified template fragments with one or more restriction endonucleases that recognize the restriction endonuclease recognition site of the primers to create overhanging termini wherein each overhanging termini is complementary to only one other overhanging termini on another fragment; and

                  d) combining the amplified and digested template fragments in a ligation  
20                   reaction to produce a directionally ordered gene, nucleic acid fragment or gene vector.

2. The method of claim 1 wherein the restriction endonuclease is at least one class IIS restriction endonuclease.

25                   3. The method of claim 2 wherein the class IIS restriction endonuclease is selected from the group consisting of: *AlwI*, *Alw26I*, *BbsI*, *BbvI*, *BbvII*, *BpmI*, *BsmAI*, *BsmI*, *BsmBI*, *BspMI*, *BsrI*, *BsrDI*, *Eco57I*, *EarI*, *FokI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MnII*, *PleI*, *SapI*, *SfaNI*, *TaqII*, *Tth111II*.

30                   4. The method of claim 1 wherein class II restriction endonuclease recognition sites, linkers, or adapters are not used to create the gene or gene vector.

5. The method of claim 1 wherein the product of the ligation reaction is introduced into prokaryotic or eukaryotic cells.

5 6. The method of claim 1 wherein at least one target nucleic acid sequence is chosen from the group consisting of : transcriptional regulatory sequences; genetic vectors; introns and/or exons; viral encapsidation sequences; integration signals intended for introducing nucleic acid molecules into other nucleic acid molecules; retrotransposon(s); VL30 elements; or multiple allelic forms of a sequence.

10

7. The method of claim 1 wherein the method is used to generate combinatorial libraries of a target sequence.

8. The method of claim 7 wherein the target sequence is part or all of a gene.

15

9. The method of claim 8 wherein the gene encodes a protein.

10. The method of claim 8 wherein the primers amplify allelic variants of part or all of a gene.

20

11. The method of claim 1 wherein the product of the ligation reaction is passed between eukaryotic cells using a virus particle, by cell fusion, or by transfection.

12. The method of claim 1 wherein the product of the ligation reaction is not introduced  
25 into prokaryotic cells.

13. The method of claim 1 further combining at least one screening or selection step to select the products of the ligation reaction.

30 14. The method of claim 1 wherein the product of the ligation reaction is mutated during passage in cells in order to generate genetic diversity.

15. The method of claim 14 wherein the product of the ligation reaction is mutated by homologous recombination during passage in cells.

16. The method of claim 1, wherein the method is used to isolate and identify regulatory  
5 sequences from a cell.

17. The method of claim 11, wherein cells containing the product of the ligation reaction are selected for enhanced biological activity.

10 18. The method of claim 17, wherein the cells containing the product of the ligation reaction are selected for tissue-specific, hormone-specific or developmental-specific gene expression.

19. The method of claim 1 wherein the product of the ligation reaction is a circularized  
15 gene vector.

20. A nucleic acid primer having a 5' and a 3' end to amplify a nucleic acid fragment for the ligation of at least two fragments comprising:

a restriction endonuclease recognition site that recognizes a restriction endonuclease,  
20 wherein the restriction endonuclease cleaves at a distance from the recognition site and creates overhanging termini;

a sequence complementary to a template sequence to be amplified to produce the nucleic acid fragment;

25 at least two nucleic acid bases positioned at the restriction endonuclease cleavage site and that form an overhanging terminus after cleavage by the restriction endonuclease, wherein the at least two nucleic acid bases are selected to be complementary to only one other overhanging terminus on another fragment of the ligation; and  
an affinity handle on the 5' end of the primer.

30 21. The primer of claim 20 further comprising an anchor to provide stability to the restriction enzyme at the restriction enzyme recognition site.

22. A method for isolating and identifying promoters comprising the steps of:

a) obtaining a vector comprising at least a portion of a promoter region from a retrovirus transposon LTR and having two non-complementary overhanging termini;

b) designing at least two PCR primers to amplify at least one region of a retro-transposon LTR from template nucleic acid to produce at least one nucleic acid fragment wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template sequence from a retrovirus transposon, and bases positioned at the restriction endonuclease cleavage site that are selected to be complementary to only one other overhanging terminus of the vector wherein the restriction endonuclease cleavage site is created from enzymatic cleavage of the fragments;

c) combining the primers with template nucleic acid and performing a gene amplification reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site;

d) digesting the amplified template fragments with one or more restriction endonuclease that recognize the restriction endonuclease recognition site of the primer to create overhanging termini; and

e) combining the amplified and digested template fragment in a ligation reaction with the vector to produce a gene vector with an intact LTR sequence.

23. The method of claim 22 wherein the template nucleic acid is DNA or RNA.

24. The method of claim 22 further comprising the step of sequencing the insert to identify the promoter sequence.

25. Promoter sequences of SEQ ID NOS:2-13 identified using the methods of claim 22.

26. The vector of SEQ ID NO:1.

Fig 1A

A.

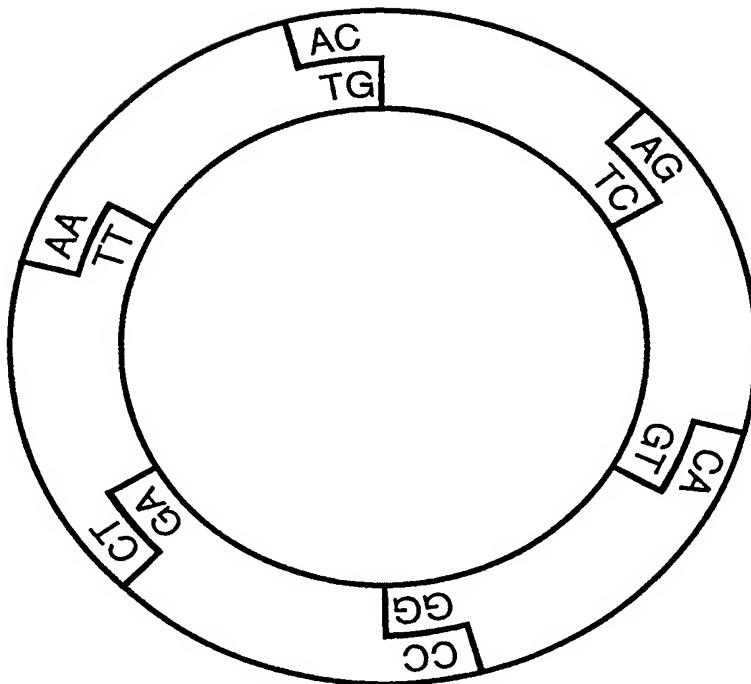
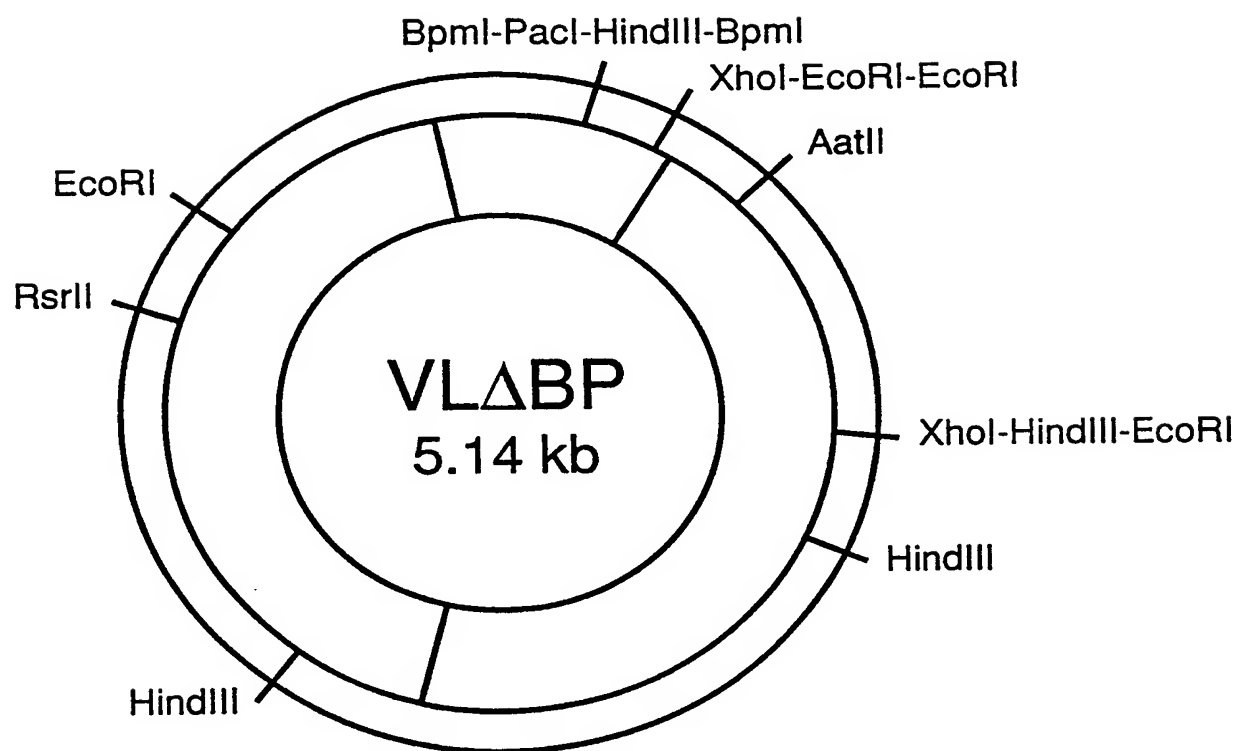


Fig 1B

B.



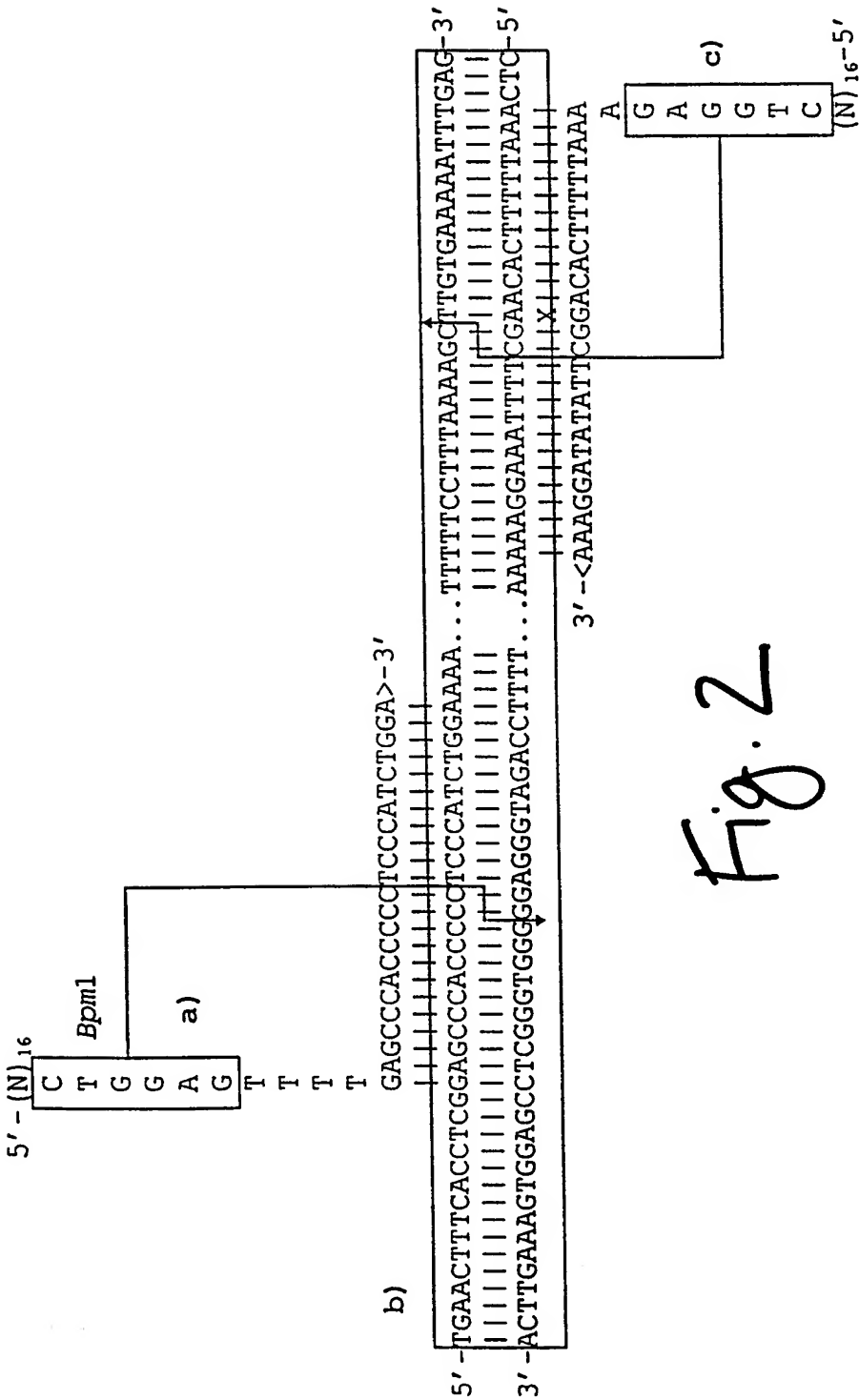
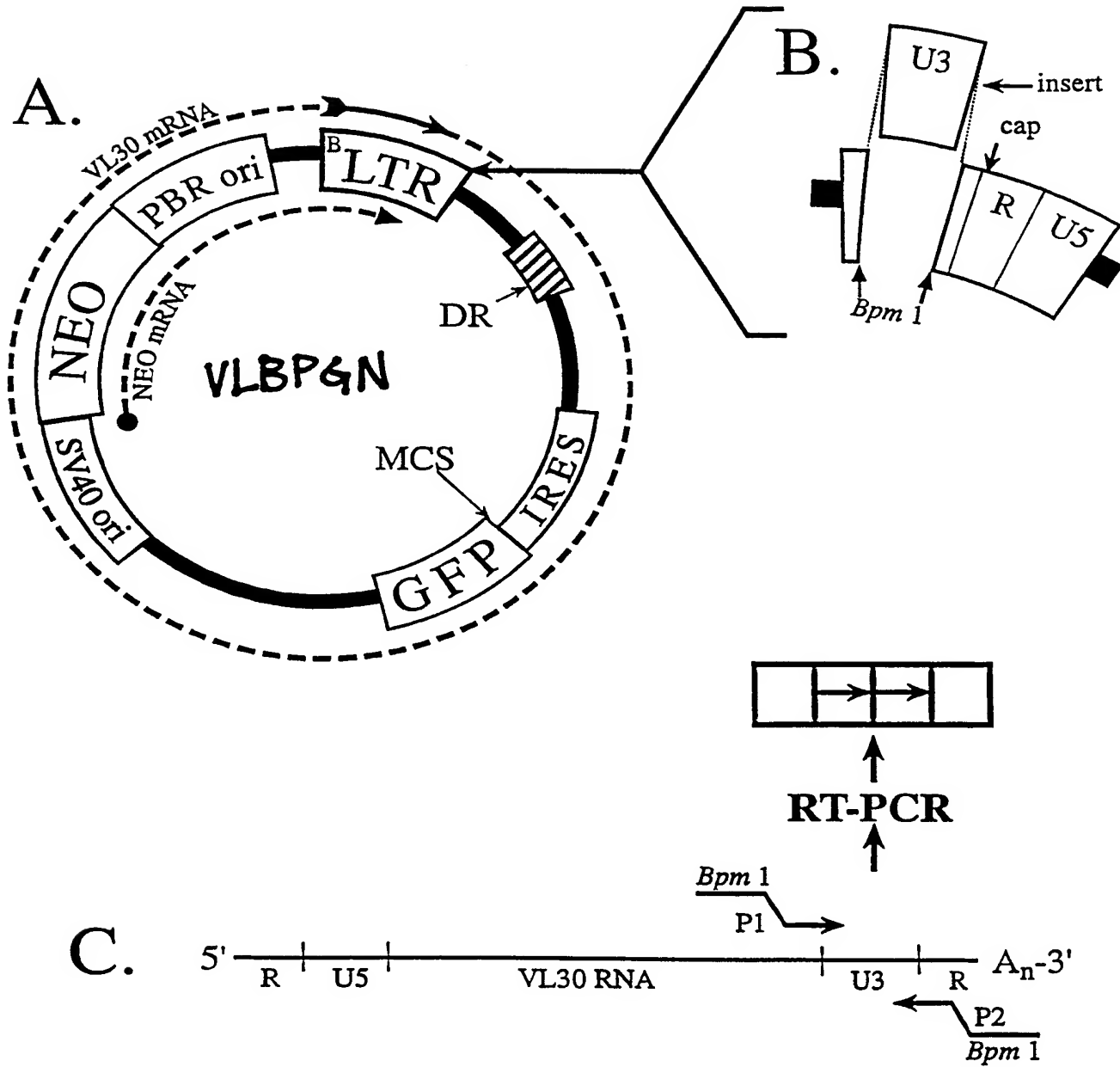


Fig. 2



Fig. 3



5/9

Fig. 4 A.

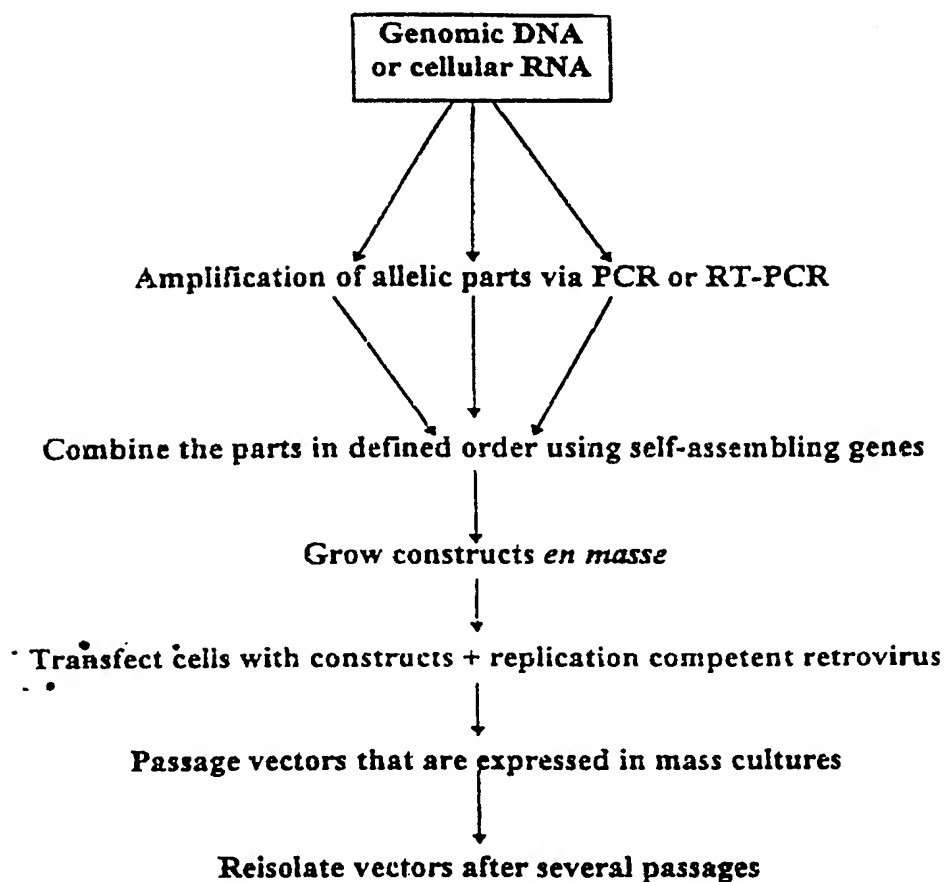


Fig 4 B.

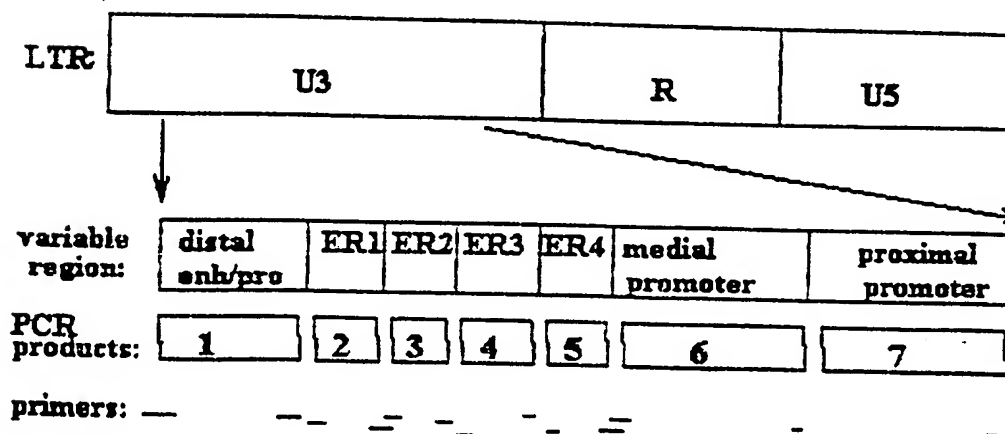


Fig. 5

```

1      CCTCCATCT AGAGGTTGTT CTGGGAACAC TCCTAACTT TTCACCCCAA AACTCCTCAC CCTAAGTTC GAAAAAAGTG TTCCAAGAAC      90
I.D.2
I.D.3

91      ATTTTGGAGA TAAAGGCCTC CTAGAACAC CTCAAAATGA CATTGCCAAT TCATAGACA TGACTCCTTA GTTACGTTAGG TTCCTTGATA      180
I.D.2
I.D.3

181      GGACATGACT CCTTAGTTAC GTAGGTTTCT TGATAGGACA TGACTCCTTA GTTACGTTAGG TTTCTTTGGT AGAATCCCT AGTGATGTAA      270
I.D.2
I.D.3

271      ACCAGGTACT CCTTAGTTAC GTAGGTTTCT TGATAGGACA TGACTCCTTA GTTACATAGA TTCCTTTGGC AGAATCCCT AGTGATGTAA      321
I.D.2
I.D.3

```

Fig. 6

1 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC 90  
4 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
5 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
6 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
7 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
8 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
9 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
10 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
11 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
12 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC  
13 CCTCCCATCT AGAGATGTGT CCTCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATGCC TGAACCTCTC ACCCTAGAGT TCGAACCCCTC

91 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA 180  
4 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
5 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
6 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
7 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
8 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
9 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
10 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
11 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
12 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA  
13 CCAACTAAAG ACTGTTCCAA GAACATTTTT GAGATAAGG CCTCTGGAA CAACCTCAGA ATGAACCGG TACATTGCCA AATAATAGGA

Fig. 7

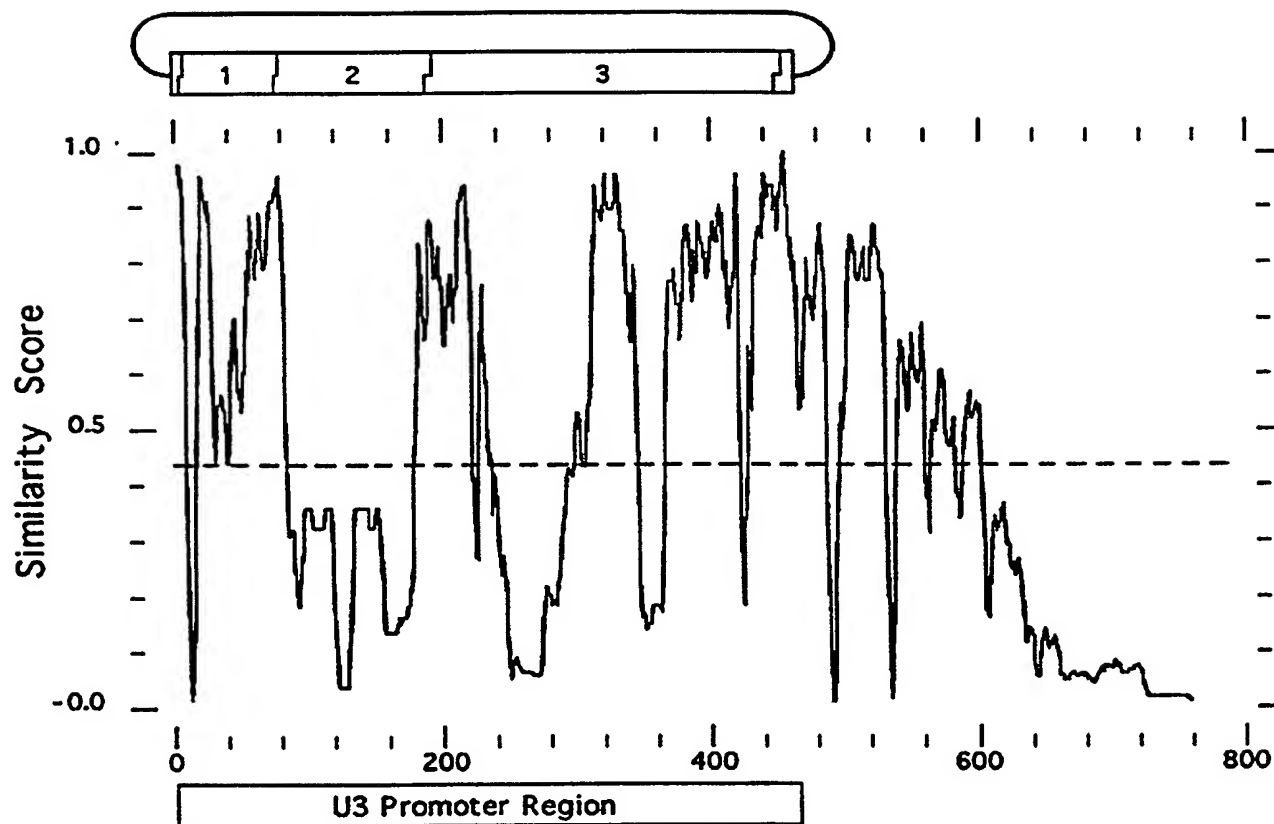
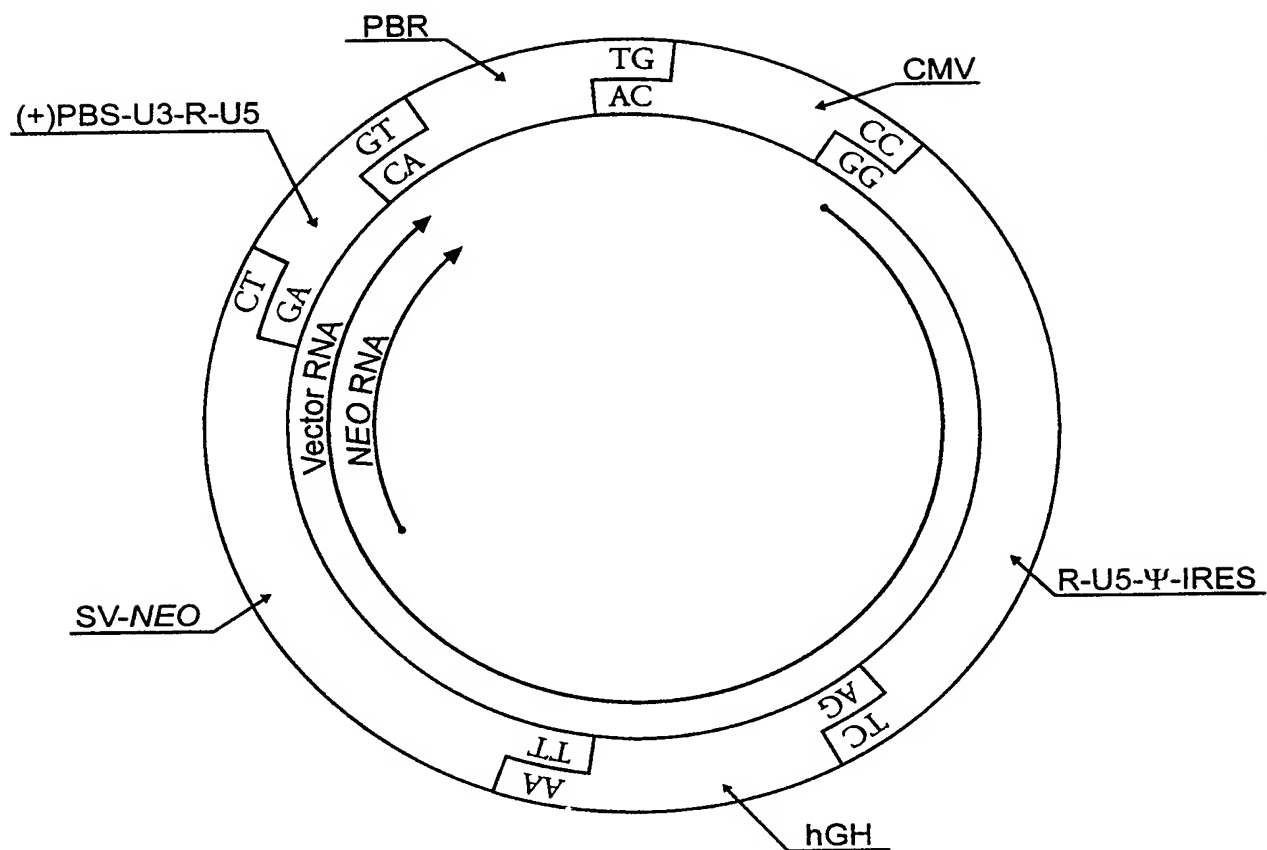


Fig. 8



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/03918

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/86 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PADGETT K A ET AL: "Creating seamless junctions independent of restriction sites in PCR cloning" GENE, vol. 168, no. 1, 2 February 1996, page 31-35 XP004042930 see the whole document	1,2, 4-14, 19-21
Y	---	3
Y	TOMIC, M. ET AL.: "A rapid and simple method for introducing specific mutations into any position of DNA leaving all other positions unaltered" NUCLEIC ACIDS RESEARCH, vol. 18, no. 6, 1990, OXFORD GB, page 1656 XP002069445 cited in the application see the whole document ---	3
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance  
"E" earlier document but published on or after the international filing date  
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
"O" document referring to an oral disclosure, use, exhibition or other means  
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.  
"Z" document member of the same patent family

Date of the actual completion of the international search

26 June 1998

Date of mailing of the international search report

09/07/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Chambonnet, F

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03918

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LEBEDENKO, E.N. ET AL.: "Method of artificial DNA splicing by directed ligation" NUCLEIC ACIDS RESEARCH, vol. 19, no. 24, 1991, OXFORD GB, pages 6757-6761, XP002069446 cited in the application see the whole document ----	1
A	CHAKRABORTY, A.K. ET AL.: "Synthetic retrotransposon vectors for gene therapy" FASEB JOURNAL., vol. 7, no. 10, July 1993, FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD US, pages 971-977, XP002029486 see the whole document ----	1
P,X	WO 97 28282 A (STRATAGENE INC) 7 August 1997	1,2, 4-14, 19-21
P,Y	see the whole document ----	3
P,X	HODGSON, C.P. ET AL.: "Self-assembling genes (SAGE) : construction of complex vectors and combinatorial libraries without sub-cloning" CANCER GENE THERAPY, vol. 4, no. 6 conf. suppl., November 1997, page s27 XP002069448 see the whole document ----	1
P,X	ZINK, A. M. ET AL.: "Transcriptional targeting with rescued LTRs : a hepatocyte promoter" CANCER GENE THERAPY, vol. 4, no. 6 conf. suppl., November 1997, page s28 XP002069449 see the whole document -----	22



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/03918

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9728282      A	07-08-1997	NONE	